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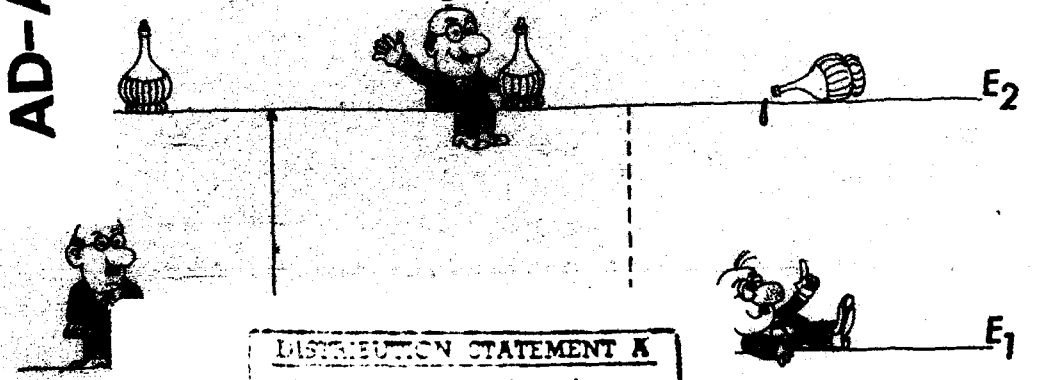

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3rd CHIANTI WORKSHOP ON MAGNETIC RESONANCE

NUCLEAR AND ELECTRON RELAXATION

SAN MINIATO (PISA), ITALY

MAY 28 - JUNE 2, 1989

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ABSTRACTS

3rd Chianti Workshop on Magnetic Resonance

NUCLEAR AND ELECTRON RELAXATION

San Miniato (Pisa) - Italy - May 28 - June 2, 1989

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3rd Chianti Workshop on Magnetic Resonance

NUCLEAR AND ELECTRON RELAXATION

San Miniato (Pisa) - Italy - May 28 - June 2, 1989

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Preface

This is now the 3rd meeting of the Chianti Workshop series. This time the Chairman of the scientific program committee is Prof. J.W.Emsley of the University of Southampton. The Chairman of the scientific program for the 3rd Workshop was designated by The International Advisory Board and approved by the partecipants at the 2nd Workshop.

The success of the present Workshop as it appears from this collection of abstracts seems great. There is a large highly selected partecipation from the advanced countries.

We have decided to maintain for the time beeing the title "NUCLEAR AND ELECTRON RELAXATION" since there is little overlap with related meetings. It appears that the meeting is becoming an international stage where scientists of different backgrounds can discuss the problems of nuclear and electronic relaxation. During this meeting will discuss organizational aspects (including the Chairman) of the 4th Chianti Workshop.

We are grateful for patronage and financial contributions to the three Tuscanian Universities, the Italian Consiglio Nazionale delle Ricerche, and the Regional Goverment as well as the Italian Chemical Society, the Italian Gruppo Discussione Risonanze Magnetiche, the Minister of Foreign Affairs, the European Research Office of the US Army and the US Navy, Bruker Spectrospin S.r.l., Varian S.p.a., Piccinotti Assistenza Tecnica, Stelar S.r.l., Istituto Guido Donegani - Montedison.

We deeply thanks the Consorzio of Chianti Classico Gallo Nero for providing the red fluid necessary to keep us in the excited state in a pleasent and happy atmosphere.

Finally, I would like to acknowledge the Cassa di Risparmio di San Miniato for allowing us to held the meeting at I Cappuccini, and Mr. C.Bertini ("Canapone"), his wife, and all the staff who will take care of us during the meeting with the usual enthusiasm and good will.

Ivano Bertini

ABSTRACTS

Synthesis of the Dilactone Derivative of GD1b Ganglioside: Structure and Conformation Elucidation by Proton N.m.r.

Domenico Acquotti, *Giovanni Fronza, **Gunther Kirschner and Sandro Sonnino

Study Center for the Functional Biochemistry of Brain Lipids, Department of Medical Chemistry and Biochemistry, Medical School, University of MIlan; *CNR Study Center for Natural Organic Substances, Department of Chemistry, Polythecnic of Milan; **FIDIA Research Laboratories, Department of Chemistry, Abano Terme (Italy).

Treatment of GD1b, ß-Gal-(1--3)-ß-GalNAc-(1--4)-{α-Neu5Ac-(2--8)-α-Neu5Ac-(2--3)}-ß-Gal-(1--4)-ß-Glc-(1--1)-Cer, with dicychlohexylcarbodi-imide in anhydrous methyl sulfoxide affords 95-98% of GD1b-dilactone. The involvement of the carboxyl groups of the two sialic acid units in the ester linkages was proved by ammoniolysis and reduction experiments, which gave ganglioside derivatives containing the amide of sialic acid and *N*-acetylneuraminulose, respectively. ^{1}H-N.m.r. spectroscopy showed that the lactone rings involved position 9 of the inner sialic acid and position 2 of the inner galactose in the ester linkages, and that the disialosyl chain is extended toward the -ß-Gal-(1--4)-ß-Glc- portion of the oligosaccharide ganglioside moiety.

^{2}H-NMR Spectra and Spin-Lattice Relaxation of Some Partially Deuteriated di-n-alkyl- and di-n-alkyloxy- azoxybenzenes

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[a] Istituto di Chimica Quantistica ed Energetica Molecolare, CNR, I-56100 Pisa, ITALY
[b] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, I-56100 Pisa, ITALY

In order to study the influence of the end chains on the orientational behaviour of mesogens, we have studied three liquid crystals (di-n-heptylazoxybenzene (I), di-n-octylazoxybenzene (II) and di-n-hexyloxyazoxybenzene (III)) which differ either in the length of the chain or in the first member of the terminal chains. These compounds were deuteriated on the aromatic core and there was also partial deuteriation on the chains. ^{2}H-NMR spectra were recorded and analysed in order to obtain orientational and structural information; deuterium spin-lattice relaxation times (T_1) were also determined for information on intramolecular and reorientational motions.

^{2}H-NMR spectra of di-n-heptylazoxybenzene, di-n-octyl-azoxybenzene and di-n-hexyloxyazoxybenzene have been recorded at different temperatures covering all the mesomorphic range of the three compounds. The data reveal a different degree of orientational order of the two rings of the aromatic core. The biaxiality of this central fragment in the three compounds is discussed. The dipolar and quadrupolar splittings measured also give information on the ring C-D bond angles if the quadrupolar parameters are assumed. The spectra show that such angles are not all equivalent, and the two rings are structurally different. The orientational behaviour of the two liquid crystals (I) and (III), which differ only in the first member of the end chains, is compared and analysed considering the different conformations of the terminal chains.

Deuteron spin-lattice relaxation times (T_1) of the different deuterons have been measured at various temperatures. Analysis of the trend of T_1 with temperature, furnishes activation energies for spin-lattice relaxation in the different mesomorphic phases. The principal relaxation mechanism is shown to be the reorientation around the long molecular axis; the correlation times for this type of motion can therefore be calculated at the different temperatures, thus giving information on the internal motion of the liquid crystal molecules both in the nematic and the smectic range. Preliminary measurements show differences between the two rings of the aromatic core. This can, in part, be justified by the structural difference and different orientational behaviour observed for the two rings from the previous analysis of the ^{2}H-NMR spectra. The trends of the T_1's for the alkyl- and alkyloxy- compounds in the liquid-crystalline phases are compared and discussed.

Relaxation studies by ^{14}N Nuclear Quadrupole Resonance: Methodology, Instrumentation, Results

Roberto Ambrosetti, Claudia Forte, Domenico Ricci
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Via Risorgimento 35, I-56100 Pisa (Italy)
Bitnet PI0313 AT ICNUCEVM

^{14}N NQR relaxation offers the experimenter some unique possibilities, since it samples the lowest range (1-3 MHz) of the energy spectral density, in the solid state. This is in a sense limitating, since the requirements and relatively low sensitivity of the technique allow only a rather limited range of pure, well crystallized, N-containing compounds to be studied. On the other hand, in favourable cases valuable information is gained. Examples will be given, taken mainly from work carried out in this laboratory.

The measured T_1 values span a wide range, from less than a ms to minutes (even days at liquid He temperature). Even at room temperature, T_1 values as long as 1-5 s are not uncommon. This reflects the "magnetic isolation" of the ^{14}N nucleus, which at first order, in zero external magnetic field, has no effective magnetic moment. This opens the possibility of exploring relatively ineffective relaxation channels, such as modulation of the electric field gradient, which may become more effective than magnetic modulation at long internuclear distances.

The measured T_1, T_2, T_2^* are the result of a multitude of effects. While this is true of any relaxation study, in the solid state the problem is outstanding. Only when a single mechanism is prevailing straightforward interpretation of the results is possible. This is the case of phenomena related to blocking of internal rotation of groups and to phase transitions.

The equilibrium-recovery dynamics for ^{14}N is intrinsically non-exponential, since three levels are involved even in an "isolated" system. So it is difficult to define a single relaxation time. This kind of problems is not unique to ^{14}N, but affects to some degree, although for different reasons, all relaxation measurements.

Finally, the instrumental requirements will be discussed, with special attention to a newly developed, PC-based data acquisition and handling system which can be of interest in a broad set of magnetic resonance studies.

Studio LOMENDOR e LOMESR di un cristallo irradiato di l-alanina: applicazione della nuova metodologia di misura dei tempi di rilassamento.

R. Angelone, C. Pinzino, R. Settambolo

Istituto di Chimica Quantistica ed Energetica Molecolare del CNR. Via Risorgimento, 35 56100 PISA.

I tempi di rilassamento elettronici e nucleari e le probabilitá di transizione del sistema elettrone-protoni metilici in un cristallo irradiato di l-alanina sono stati determinati in un intervallo di temperatura compreso da 200 a 350ºK, usando le potenzialitá delle nuove metodologie spettroscopiche di multipla irradiazione a risposta non lineare denominate LOMENDOR e LOMESR (1).

Una più completa trattazione teorica della metodologia LOMENDOR è stata formulata allo scopo di renderla applicabile al sistema in studio.

La tecnica sperimentale è stata semplificata e sviluppata in alcuni dei suoi aspetti strumentali.

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2D ^{1}H-NMR analysis of spatial structure of scorpion insectotoxin. Compl relaxation matrix and DISMAN calculations.

A.S. Arseniev, V.N. Maiorov, A.L. Lomize, A.G. Sobol, I.V. Maslennikov and V.F. Bystrov

Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Ul.Miklukho-Maklaya 16/10, 117871 Moscow, USSR

Overall folding of insectotoxin (35 amino acid residues, four disulfide bridges) from the venom of scorpion *Buthus eupeus* was revealed previously based on the NMR data (A.S. Arseniev et al. (1984) FEBS Lett. **165**, 57). At present we continue the refinement of the structure. The problem of protein 3D structure calculation is decomposed into two subproblems. At the first stage local spatial structure of each sequential dipeptide fragments are confined in the conformational space of torsional angles according to the experimental NOE intensities by complete relaxation matrices approach. The confined conformational space of the fragments is further restricted by use of the vicinal spin-spin coupling constants. At the second stage the obtained information on the local structure is employed to constrain the variable torsion angles in the process of overall 3D structure calculation by DISMAN algorithm (W. Braun and N. Go (1985) J. Mol. Biol. **186**, 611). It is found that introduction of local structure constraints significantly reduce the conformation space of the molecule. This results in efficiency enhancement of DISMAN calculations and increasing accuracy of 3D structure determination. The overall fold of insectotoxin includes α-helix (Met 12 - Cys 20) and antiparallel β-structure (Asn 23 - Asn 34) with reverse turn (Phe 27 - Gln 30).

The ^{1}H NMR Parameters of Magnetically Coupled Dimers. The Fe_2S_2 Proteins and Cu_2Co_2 SOD as Examples

Lucia Banci, Ivano Bertini and Claudio Luchinat

Department of Chemistry, University of Florence and Institute of Agricultural Chemistry, University of Bologna, Italy.

The interaction energy between nuclei and magnetically coupled electrons is discussed in terms of the Heisenberg formalism. Simplified formalisms are proposed in order to find coefficients which relate the T_1 and isotropic shift values of a nucleus in a monometallic system with those of a nucleus in a dimetallic magnetically coupled system. Another aspect which is addressed along this research is that of providing some guidelines in the understanding of the effect on electron relaxation times when magnetic coupling interactions occur in a dimeric system.

The theory is tested for the cases of Cu_2Co_2SOD, $Fe^{III}Fe^{III}S_2$, and $Fe^{III}Fe^{II}S_2$ ferredoxins with quite satisfactory succcess.

at the temperature of the EPR experiment. As far as nuclear relaxation at room temperature is concerned this approximation may often break down. As a result, $S>1/2$ systems can only be treated by including in the spin Hamiltonian the $S \cdot D \cdot S$ term. It can be shown that, under these conditions, the actual g–anisotropy present in the Zeeman term introduces only a small further perturbation and can be neglected.

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3) I. Bertini, C. Luchinat "NMR of Paramagnetics Species in Biological Systems" Benjamin Cummings, Boston 1986

4) J. Kowalewski, L. Nordenskiold, N. Benetis, P. O. Westlund, Progr. NMR Spectr. 17, 141 (1985) and refs. therein.

5) K. V. Vasavada, B. D. Nageswara Rao, J. Magn. Reson. 81, 275-283 (1989)

MIXED LIGAND COMPLEXES OF CU(II)-1,10-o-PHENANTHROLINE AND ITS ANALOGUES CHARACTERIZED BY COMPUTER AIDED ESR SPECTROSCOPY

Riccardo Basosi, Rebecca Pogni and *Yan Yang
Department of Chemistry - University of Siena - Italy

An extensive reinvestigation based on computer aided ESR spectroscopy, for Cu-1,10-o-Phenanthroline (CuOP) and its analogue Cu-2,9-Dimethyl-1,10-o-Phenanthroline (CuDMP) was performed in liquid and solid phases under a large range of pH, temperature and molar ratio conditions. Equilibria involved in solution and coordination behaviour in function of pH have been characterized by a careful analysis of magnetic ESR parameters. Isotopic selective enrichment and computer aided ESR spectroscopy have been used for precise determination of Spin Hamiltonian parameters and correlation times.

Cu-1,10-o-Phenanthroline (CuOP) and its analogue Cu-2,9-Dimethyl-1,10-o-Phenanthroline (CuDMP) have been reported to possess very different chemical and biological characteristics despite being conformational homologous. The 2,9DMP form possesses a marked specificity and stereochemistry that confers upon the bidentate cupric chelate potent oxidative properties. This could be attributed to the steric hindrance to normal planar configuration caused by the presence of methyl groups proximate to the metal coordination site, a feature not shared by 1,10-Phenanthroline. A structure-activity relationship is proposed on the basis of difference in magnetic parameters and consequent evaluation of molecular orbital coefficients K and α^2 under assumption of an effective D_{4h} tetragonally distorted octahedral symmetry.

Our results are in favour of two distinct structures for 1,10-o-Phen and 2,9-DMP cupric complexes in solution: a tetragonal structure probably with rhombic distortion for OP and a flat tetrahedral structure for DMP. The presence of one or two ligands coordinating the metal ion does not seem to play a determinant role in the structural arrangement. On the contrary the presence of substituent Methyl groups in position 2- and 9- is fundamental for explaining the striking differences shown by the analogue compounds and can reconcile the manifestation of unexpected properties.

*on leave from school of Pharmacy, West China University of Medical Sciences, Chengdu, The People's Republic of China.

Heteronuclear relaxation studies of proteins

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Steven W. Sparks[&] and Dennis A. Torchia[&]

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^{15}N labeling of cloned proteins is a relatively simple and inexpensive procedure which permits the measurement of relaxation properties of these backbone nuclei. We have developed sensitive and convenient two-dimensional NMR procedures for measuring both the T_1 and T_2 values of these nuclei, providing insight in the protein backbone dynamics.

More subtle relaxation effects will also be discussed. For example, it is shown that the relaxation rate of heteronuclear ^{15}N-^{1}H multiple quantum coherence is significantly longer compared to the ^{1}H T_2, providing an opportunity to increase resolution in 2D ^{1}H NMR spectra and to measure ^{1}H-^{1}H J couplings in proteins of up to 20 kD.

As is well known, cross correlation between heteronuclear dipolar coupling and ^{15}N chemical shift anisotropy can give rise to differential decay rates of heteronuclear doublet components. In addition, we have observed a cross correlation effect between ^{1}H-^{1}H dipolar couplings and ^{15}N CSA.

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Title:"A Theoretical NMR Lineshape Study of Quadrupolar Ions, I=3/2, in Chemical Exchange Between Intracellular Sites"

Abstract

Quadrupolar NMR studies of chemical exchange between different sites in an intracellular environment is a complex and interesting problem, both experimentally and theoretically. This presentation will mainly treat the case where there is a two site chemical exchange of sodium ions (I=3/2), between a bounded isotropic site in slowmotion regime, for example the ions associated to a protein, a macromolecule or an intracellular membrane, and a free, fast modulated, bulk site, where the ions are fully hydrated, when the chemical exchange time is in the same range and shorter than the correlation time of the site in slowmotion. The intricate part of this problem is how the chemical exchange rate will disturb the correlation time of the bounded site and in what way it will affect the lineshape under these circumstances.

Our calculations are based on the semi–classical Liouville equation.

^{1}H RELAXATION IN HYDRIDO CARBONYL COMPOUNDS

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Nuclear magnetic resonance spin-lattice relaxation times (T_1) measurements can be used, in principle, without the need of any other data, to obtain a number of parameters tha can provide useful information about molecular structure and molecular geometry[1].
The inversion-recovery tecnique[2] has been employed to measure proton T_1 on degassed solution of $[HRe(CO)_5]$ (I), $[HRe(CO)_4(PPh_3)]$ (II) and $[H_2Re_2(CO)_8]$ (III) in toluene-d_8. The temperature and the field strenght were varied in order to ascertain the main relaxation mechanism. The number of the variable delays used in each experiments ranged typically from 13 to 20; relaxation times were calculated both graphically and with a three parameter fitting routine provided by the spectrometer manifacturer. The experimental results show the following features.
i) T_1's decrease on decreasing the temperature for all the three compounds. ii) Compounds (II) and (III) show at low temperature and higher field (200 MHz) a minimum indicating that dipole-dipole relaxation is the main relaxation pathway. iii) Short relaxation times (less than 100 ms) were observed at low temperature at 80 MHz for (II) and (III) outlining that care must be taken to define the conditions in which short relaxation times can be considered as probes of the existence of dihydrogen coordinated to the metal; iv) For compound (I) T_1 are longer than the ones measured for the other two compounds due to the absence of a source of efficient dipolar relaxation (^{1}H or/and ^{31}P) within the molecule and only dipolar coupling with Re allows relaxation; as discussed by Farrar and Quinting[3] for $[HMn(CO)_5]$ the scalar coupling with a quadrupolar nucleus affects the transverse relaxation times (T_2) . The difference between T_1 and T_2 for hydrogen is a function of $T_1(Re)$ and $J(H-Re)$ according to

$$1/T_2(H) - 1/T_1(H) = (35/3)\pi^2 J(H-Re)^2 T_1(Re)$$

The combined measurements of T_1 and T_2 for the proton and an estimate of the rotational correlation time in $[HRe(CO)_5]$ allow therefore the calculation of $T_1(Re)$ and $J(H-Re)$.

---

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2) Levy, G. C. and Peat, I. R., J. Magn. Reson, 18, 500, (1975).
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^{1}H NMR STUDIES OF THE OXIDIZED AND PARTIALLY REDUCED 2(4Fe–4S) FERREDOXIN FROM CLOSTRIDIUM PASTEURIANUM

Ivano Bertini,[a] Fabrizio Briganti,[a] Claudio Luchinat,[b] and Andrea Scozzafava[a]

Contribution from the [a]Department of Chemistry, University of Florence, Via Gino Capponi 7, 50121 Florence, Italy, and [b]Institute of Agricultural Chemistry, University of Bologna, Viale Berti Pichat 10, 40127 Bologna, Italy.

During the last years iron–sulfur proteins have attracted the interest of researchers owing to their role in *electron–transfer processes* during mitochondrial respiration, nitrogen fixation, and photosynthesis.[1] These systems are able to reversibly transfer one or more electrons and at least one of their oxidation states is paramagnetic; the presence of a paramagnetic center makes them quite suitable for NMR spectroscopy.[2] ^{1}H NMR spectra of the oxidised ferredoxin from *Clostridium pasteurianum* which contains two weakly paramagnetic $Fe_4S_4^{2-}$ clusters, coordinated to the protein through cysteinyl sulphurs, have been recorded. Use of suitable pulse sequences (Super WEFT and MODEFT)[2] has allowed to reveal signals of protons feeling the paramagnetic clusters well inside the diamagnetic part of the spectrum. Despite the protein has a paramagnetism roughly corresponding to a single unpaired electron, and the shifts are relatively small ($\lesssim$ 18 ppm) expecially with respect to the analogous $(2Fe–2S)^{3-}$ system,[3] the T_1^{-1} values are very long (5–15 ms). This is possibly to be ascribed to a long electronic relaxation rate which is unexpected in metal clusters. Indeed, iron (II) is expected to have large electronic relaxation rates as in FeS_4^{2-} or $Fe_2S_2^{3-}$ and the electrons of iron (III), when coupled with iron (II), are expected to relax at the same rate.[3] The particular nature of the chemical bonds in the clusters, i.e. the large delocalisation, makes the above description unrealistic and may account for the small electronic relaxation rates. ^{1}H NOE have been detected for β–CH_2 cysteinyl geminal protons owing to their short reciprocal distances, despite the short T_1. This allowed us to assign the pairs of geminal protons, and to try to test the shift dependence on the dihedral angles between the Fe–S–C and S–C–H planes with respect to the structure of the analogous ferredoxin from *Peptococcus aerogenes*. In the presence of fully reduced, one electron oxidised, and fully oxidised species, ^{1}H saturation transfer experiments are possible and they allow to set the lower limit of the electron transfer rate ($6x10^2$ s^{-1}) and provide a tool for the assignment of all the signals of the various species.

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(2) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Molecules in Biological Systems*; Benjamin/Cummings Menlo Park, CA, 1986.

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NUCLEAR RELAXATION IN PARAMAGNETICS SYSTEMS

Ivano Bertini, Claudio Luchinat, Marcello Mancini, Gabriele Spina
and Paola Turano

Contribution from the Department of Chemistry, University of Florence, Florence, Italy; the Department of Physics, University of Florence, Florence, Italy; the Institute of Agricultural Chemistry, University of Bologna, Bologna, Italy.

The general problem of nuclear relaxation in paramagnetic macromolecules is discussed in terms of the appropriate spin Hamiltonian for paramagnetic metal ions and of its effects on the electron nucleus coupling. Formally, the cases of interest are those for which the correlation time for the electron–nucleus interaction is shorter than the rotational correlation time of the molecule.[1–3] It is assumed that electron relaxation can be described in the Redfield limit.[4] Often the correlation time in macromolecules is the electronic relaxation time itself. Under these conditions the presence in the spin Hamiltonian of terms like g–anisotropy, hyperfine coupling with the metal nucleus, and zero field splitting becomes relevant and must be properly taken into account.

In particular, the cases of g–anisotropy and zero field splitting are re–examined to ascertain whether and to what extent the effect of zero field splitting can be mimicked by a fictitiously large g–anisotropy.[5] It is well known that EPR spectra of $S>1/2$ species can be reproduced by fictitious spin Hamiltonians with $S'=1/2$. This usually leads to large anisotropies in the corresponding g tensor. Physically, the resulting g tensor represents the Zeeman splitting anisotropy of the ground state Kramers' doublet, and is a good approximation for the whole spin system when the latter is the only energy level populated

THE ^{1}H NMR SPECTRA OF THE Co_4S_{11} CLUSTER IN METALLOTHIONEINS.

Ivano Bertini, Claudio Luchinat, Luigi Messori and Milan Vasak.
Contribution from the Department of Chemistry, University of Florence, Florence, Italy; the Institute of Agricultural Chemistry, University of Bologna, Bologna, Italy; and the Biochemical Institute, University of Zurich, Zurich, Switzerland.

Metallothioneins are a class of low molecular weight, single chain proteins capable of binding seven bivalent metal ions bound in two independent clusters of three and four metal ions, respectively. The cobalt(II)$_7$ derivative gives a surprisingly well resolved ^{1}H NMR spectrum characterized by more than 20 isotropically shifted signals spread over a region spanning between 300 and –100 ppm. The signals have been unequivocally assigned to the Co_4 cluster, by comparing the spectra of the Co_7 derivative and the Cd_4Co_3 derivative; the Co_3 cluster in the Cd_4Co_3 derivative does not give sharp isotropically shifted signals probably due to exchange phenomena. Most of the far isotropically shifted ^{1}H NMR signals of the Co_4 cluster arise from the β–CH_2 protons of the eleven cysteines involved in metal coordination; some of the less shifted signals, both up and downfield, might be assigned to Cys α–CH protons.

We have extended the theory for the electron–nucleus interaction in exchange–coupled dimetallic systems to the case of a 4–metal cluster in order to

develop a theoretical model for the experimental ^{1}H NMR spectra. A model is obtained that qualitatively rationalizes the isotropic shifts pattern and their temperature dependence in terms of five large and one essentially zero J values for the six exchange coupling constants operative in the 4–metal cluster. These findings are consistent with the results from the X–ray and solution structures of the protein.

Preliminary ^{1}H NOE studies permit to determine the spatial connectivities existing among the isotropically shifted signals and are of great help for the assignment of the ^{1}H NMR spectra. Geminal connectivities between the protons of the same methylene group have been unambiguously identified for at least 9 out of the 11 cysteines of the four metal cluster; further connectivities have been found between protons of different methylene groups. With the aid of computer graphics analysis of the cluster, it is possible to propose a preliminary assignment of some ^{1}H NMR signals and to start mapping the chromophore.

PROTON NMR STUDIES OF LANTHANIDE TRANSFERRINS.

Ivano Bertini, Claudio Luchinat, Luigi Messori, Mario Piccioli
and Andrea Scozzafava.

Contribution from the Department of Chemistry, University of Florence, Florence, Italy, and the Institute of Agricultural Chemistry, University of Bologna, Bologna, Italy.

Transferrins are an important class of proteins implicated in iron metabolism in mammalians. In order to investigate the structural details of the two metal binding sites, we have replaced the native iron(III) with different lanthanides which behave as excellent shift reagents. The mono and bisderivatives of human serum transferrin with ytterbium, thulium, dysprosium and terbium produce well resolved proton NMR spectra showing several isotropically shifted signals spread between 100 and – 100 ppm. The ^{1}H NMR spectra of these derivatives allow a quite exhaustive description of the interaction between lanthanides and transferrin. In particular structural inequivalence between the two metal sites as well as sequential binding have been clearly demonstrated. Also, the conformational effect of sodium perchlorate has been investigated.

Isotropic shifts induced by lanthanides are mainly dipolar in origin and depend on the metal proton distance and on a geometrical factor. Therefore, in principle, analysis of the dipolar shifts permits to get structural information on the system. We have attempted such an analysis using the recent X–ray crystallographic data of rabbit serum transferrin; preliminary information on some structural features of the metal chromophore in solution is obtained.

EPR AND ELECTRON SPIN ECHO STUDIES OF THE MANGANESE SITES ASSOCIATED WITH PHOTOSYNTHETIC OXYGEN EVOLUTION.* R. David Britt, Victoria deRose, S. L. Dexheimer, K. Sauer and Melvin P. Klein, Chemical Biodynamics Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA. 94720, USA. [M. P. Klein on Leave at: Institut für Molekülphysik, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33].

A cluster of four Mn atoms is associated with the process by which photosynthetic organisms store the energy of four sequential photons in five intermediate states, S_0-S_4, and ultimately transfer the oxidizing equivalents to two molecules of water to yield molecular oxygen. Previous work in our laboratory has shown that the manganese atoms occur, minimally, as binuclear di-μ-oxo bridged dimers with low-Z ligands that undergo photo-induced oxidation.[1,2] Illumination of dark-adapted preparations of the Oxygen Evolving Complex (OEC) at 190K produces the S_2 state of the photo cycle which exhibits a complex EPR signal near g=2 containing between 16 and 19 hyperfine lines that is assignable to an exchange coupled pair of Mn.[3] Comparison with models suggests a Mn(III,IV) species. Illumination at 140 K also brings about Mn oxidation but yields an EPR signal at g=4.1.[4] Reports of an apparent non-Curie behavior of the g=2 signal led to the suggestion that it arose from an excited S=1/2 level of a quartet whose ground S=3/2 level led to the g=4.1 signal. [5] **1.** We have determined the relaxation rates and signal amplitudes of the g=2 signal by electron spin echo (ESE) detected EPR at 1.5K and 4.2K. The signal intensity is exactly proportional to the inverse temperature establishing that the S=1/2 signal is a ground state. The T_1 increases from several msec to several hundred msec suggesting that saturation of the CW EPR spectrum was the origin of the apparent non-Curie behavior. **2.** Based on our X-ray absorption edge measurements,[2] we believe that the light advances correspond to one-electron oxidations of the Mn. Thus we have sought signals from non-Kramers species in states oxidized or reduced by one electron from that yielding the S=1/2 signal. Using a bimodal cavity we have observed a new signal near g=4.8 for samples in the dark-adapted S_1 state, when the microwave H_1 field is parallel to the Zeeman field. The new signal may arise from so-called $\Delta M=2$ transitions within an S=1 manifold or similar transitions within an S=2 manifold. Upon illumination the intensity of the new signal decreases while that of the S_2 state signal increases proportionately. **3.** As histidine frequently contributes ligands to metals in metalloproteins and because there are a pair of highly conserved histidine residues in the proteins that putatively bind the manganese, it has been assumed that these residues ligate the Mn. To test the validity of this hypothesis we have grown the oxygenic photosynthetic cyanobacterium *Synechococcus sp* on media containing solely ^{14}N or ^{15}N as NO_3^- and compared the ESE nuclear envelope modulation frequencies of the respective preparations. The frequencies of the major features are essentially identical in the two samples thus establishing that nitrogen and hence histidine is not liganded to Mn.

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MOLECULAR DYNAMICS OF PHOSPHOLIPID BILAYERS FROM DEUTERIUM NMR SPECTROSCOPY

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The interpretation of longitudinal relaxation rates (R_{1Z}) of phospholipid bilayers can yield information about the molecular dynamics of the molecules comprising the bilayers over a range of different time-scales. We have performed measurements of R_{1Z} of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) with perdeuterated and specifically deuterated acyl chains in multilamellar dispersions as well as in small unilamellar vesicles. Nine magnetic fieldstrengths were used, corresponding to Larmor-frequencies (ω_0) ranging from 2.5 MHz to 61.4 MHz. The R_{1Z} data can be approximated by a $(\omega_0)^{-1/2}$ dependence; fits to $(\omega_0)^{-1}$ or two-step Lorentzians are less satisfactory. Profiles of the R_{1Z} rates of DMPC as a function of the deuterated chain segment position exhibit a square-law dependence on the corresponding order profiles. The relaxation may involve fast motions, including trans-gauche isomerizations and rotational diffusion of the chains superimposed on slower motions due to collective bilayer disturbances (order-director fluctuations).

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ENDOR STUDY OF THE METHYL DYNAMICS IN SINGLE CRYSTALS
OF 4-METHYL-2,6-DI-TER-BUTHYLPHENOL

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The dynamics of the methyl group in free radicals in solids is determined by a threefold symmetry potential. At high temperature,the methyl rotation can be described as an hindered diffusion process,equivalent to jumps between the allowed equilibrium positions. The activation energy of the motion is equal to the barrier height between the potential wells..At very low temperarures,the methyl rotation is due to a quantum mechanical tunneling process through the potential barrier. This process can be considered by introducing in the spin hamiltonian an exchange term between the methyl proton spins,proportional to the amplitude of the tunneling splitting J_0 :

$$H_{eff} = \nu_e S_z - \nu_n \sum_j I_{jz} + \sum_j \mathbf{S} \cdot \mathbf{T}_j \cdot \mathbf{I}_j - 2J_0 \sum_{j<k} \mathbf{I}_j \cdot \mathbf{I}_k$$

The methyl quantum tunneling in free radicals optained by γ irradiation of molecular crystals can be studied by EPR and ENDOR spectroscopies. A single crystal of 4-methyl-2,6-di-ter-buthylphenol has been γ irradiated and ENDOR spectra have been recorded in the temperature range of 5-200 K. At high temperature,the hindered diffusion strongly influences the ENDOR enhancement. At low temperature,the ENDOR frequencies are shifted,because of the tunneling effect. From these two processes,the barrier height of the rotational potential can be independently determined.

Accurate determination of cross-relaxation and cross-correlation terms by NMR multipulse techniques.

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It is well known that nuclear spin cross-relaxation and cross-correlation terms provide an unambiguous means to derive dynamical and/or structural information because they arise from a single and well-characterized contribution. These quantities are especially interesting when they are frequency dependent due to the presence of a motion outside the extreme narrowing range. The following parameters will be considered: homonuclear and heteronuclear dipolar cross-relaxation terms, and dipolar-CSA (Chemical Shift Anisotropy) interference (cross-correlation) terms. One-dimensional pulse sequences aiming at an accurate determination of these parameters will be described. For the dipolar cross-relaxation terms, these sequences rest on the selective (possibly partial) inversion of the magnetization of one partner with emphasis put on the initial conditions prior to the evolution period. The dipolar-CSA interference term leads to the development of the longitudinal two-spin order $<2\hat{I}_z^A I_z^X>$ after the inversion of either spin A or spin X. A dedicated pulse sequence is able to convert $<2\hat{I}_z^A I_z^X>$ into observable one- quantum coherences while rejecting all other contributions. Again, initial conditions prior to the evolution period have to be accurately characterized. Initial slopes provide the CSA-dipolar spectral density at the

These techniques have been applied to a dynamical study of the aromatic moiety of micellized surfactants. ^1H-^1H and ^1H-^{13}C cross-relaxation terms, and CSA(^{13}C)-dipolar(^1H-^{13}C) interference terms have been measured at different frequencies. Results will be briefly discussed within the framework of the "two(three)-step model" in terms of fast and slow motions, and of order parameters.

NUCLEAR RELAXATION STUDIES ON MICELLAR SOLUTIONS AND LYOTROPIC LIQUID CRYSTALS.

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The nuclear magnetic resonance and relaxation provide a wealth of information on the conformations and dynamics of the components of micellar solutions and lyotropic liquid crystals. Examples are taken from recent studies on small single and double tailed surfactants (alkylammonium halides and alkylphosphates) to give a survey of the main applications of these techniques to lyotropic systems.

The proton relaxation in a wide frequency range (6-500 Mhz) has proved useful for determining the micellar size in pyridinium octylphosphate aqueous solutions. The aggregation number is concentration dependent and presents a large dispersity. Pulsed field gradient spin-echo experiments show a rapid decay of the monomer concentration above the CMC and provide the binding coefficient of the counterion to the micelle. The reorientational and internal motions of the surfactant have been investigated by means of the magnetic field dependence of the ^{31}P and ^{13}C relaxation rates. The relaxation data have been analyzed using the two step model where the spectral densities are the sum of two terms corresponding to the overall and to the segmental motions, respectively. A more detailed interpretation of these data, involving explicitly the trans-gauche isomerization rates and the conformer populations has also been proposed. Similar work has been done on the thermotropic and lyotropic lamellar phases of the same surfactant. The order parameters of C-H bonds obtained from the ^{13}C dipolar splittings are comparable to those derived from the ^{13}C relaxation in micellar solutions.

The double tailed surfactants with C4 to C10 chains give rise to highly ordered lamellar phases ($0.8 < S_{mol} < 0.9$) which have been investigated by ^{2}H, ^{13}C, ^{31}P or ^{14}N NMR. In these phases, the deuteron quadrupolar splittings as well as the ^{13}C relaxation enhanced by a paramagnetic ion bound to the polar head have shown that the number of accessible conformers is drastically reduced by steric constraints. The magnetic field and angular dependences of the ^{31}P relaxation in protonated and fully deuterated samples allow an estimate of the contributions of the rotational diffusion and of the director orientation fluctuations. Among these surfactants, the di-2-ethyl-hexylphosphate give rise to reversed micelles in solvents like benzene and cyclohexane. The density distribution of alkyl chain segments as well as the extent of the solvent penetration in the surfactant layer have been calculated from the ^{13}C and ^{31}P paramagnetic relaxation rates.

NMR INVESTIGATION ON PURINE NUCLEOTIDES USING Cr(III) PROBES. MOLECULAR MECHANICS INVESTIGATION ON Cr(III)-, Co(III)-PYROPHOSPHATE, -TRIPHOSPHATE AND -NUCLEOTIDE COMPLEXES.

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The ^1H NMR relaxation effects produced by paramagnetic Cr(III) complexes on nucleoside 5'-mono- and -triphosphates in D_2O solution at pH*=3 were measured. The paramagnetic probes were $[Cr(III)(H_2O)_6]^{3+}$, $[Cr(III)(H_2O)_3(HATP)]$, $[Cr(III)(H_2O)_3(HCTP)]$ and $[Cr(III)(H_2O)_3(UTP)]^-$, while the matrix nucleotides (0.1 M) were H_2AMP, $HIMP^-$ and H_2ATP^{2-}. For the aromatic base protons, the R_{2p}/R_{1p} ratio was always below 2.33 so the dipolar term predominates. A larger relaxation effect was found for the purine H(8) over H(2) signals with the $[Cr(III)(H_2O)_6]^{3+}$ probe, while the effect on H(2) signal was larger with all the other Cr(III) probes. The relaxation effects found for the H(8) signal of the $[Cr(III)(H_2O)_6]^{3+}/(H_2ATP^{2-}$ (0.1M), Mg^{2+} (0.1M)) system was smaller than that measured in the absence of the alkaline earth cation while the relaxation effect on the H(2) signal was enhanced.
Molecular mechanics computations were carried out on the $[Cr(III)(H_2O)_4(HPP)(\alpha,\beta)]$, $[Cr(III)(NH_3)_4(HPP)(\alpha,\beta)]$, $[Co(III)(NH_3)_3(H_2PPP)(\alpha,\beta,\gamma)]$ and $[Co(III)(NH_3)_4(HPP)(\alpha,\beta)]$ complexes. The calculated energy-minimized geometries are in good agreement with the crystal structures. Molecular mechanics calculations were also used to asses the geometry of the inner sphere $[Cr(III)(H_2O)_6]^{3+}$ and $[Cr(III)(H_2O)_3(HATP)(\alpha,\beta,\gamma)]$ complexes as well as the structures of the outer sphere $[Cr(III)(H_2O)_6]^{3+}$-(H_2AMP) and $[Cr(III)(H_2O)_6]^{3+}$- $(HIMP)^-$ species. The gas-phase structure of the $[Cr(III)(H_2O)_3(HATP)(\alpha,\beta,\gamma)]$ complex shows the existence of a hydrogen bond interaction between a water ligand and the adenine N(7). The metal center has an almost regular octahedral coordination geometry. The structures of the two outer sphere species reveal that the phosphate group interacts strongly with the hexa-aquochromium probe. In both complexes, the nucleotides have a similar "anti" conformation around the N(9)-C(1') glycosidic bond. For the $(HIMP)^-$ complex, strong hydrogen bond interactions exist between the water ligands and the inosine N(1) and O(6) atoms. The Cr-H(8) and Cr-H(2) distances revealed by the energy-minimized geometries for the two outer sphere species were used to calculate the R_{1p} values for the H(8) and H(2) signals. A comparison between the calculated and observed R_{1p} values, .70(c), 1.04(ob) (H(8)) and 0.06(c), 0.35(ob) (H(2)) for H_2AMP, and 4.76(c), 4.53(ob) (H(8)) and 0.16(c), 0.27(ob) (H(2)) for $HIMP^-$, respectively, shows that a semiquantitative rationalization of the relaxation effects can be obtained with molecular mechanics computations.

Relaxation and Coherence Transfer

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Many modern NMR techniques are based on the phenomenon of coherence transfer, i.e. they involve a transfer of transverse magnetization from one transition to another. Although two-dimensional methods such as correlation spectroscopy and multiple-quantum NMR are primarily used to explore networks of coupled spins, they can also be useful to investigate subtle relaxation effects. Thus it is possible to disentangle various pathways of longitudinal relaxation in coupled spin systems by investigating the multiplet fine-structure in two-dimensional exchange spectra ('small-flip angle NOESY'). Coherence transfer can also serve to highlight the existence of multi-exponential decays, both in transverse and longitudinal relaxation. Multi-exponential behaviour can arise because of cross-correlation between pairs of dipole-dipole interactions. In systems containing three or more spins with $I = ½$ in isotropic solution, the Zeeman terms not only couple with each other through cross-relaxation rates, but cross-correlation may lead to a partial conversion of Zeeman magnetization into *longitudinal three-spin order*. This distribution of eigenstate populations can be monitored by triple-quantum filtered two-dimensional exchange spectroscopy (TQF-NOESY). The initial build-up rate is solely determined by cross-correlation spectral densities which, if the molecular motion is isotropic, depend on second-order Legendre polynomials of angles subtended by pairs of internuclear vectors.

^{31}P NMR RELAXATION FEATURES OF PHYTIC ACID, ITS PARAMAGNETIC COMPLEXES WITH Gd(III) OR Fe(III) AND ITS ADDUCT WITH LYSINE

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Phytic acid (myoinositol hexaphosphate) has been attracting a great piece of biomedical interest as a carrier for the liver in nuclear medicine and as a good complexing agent.

Here we present a thorough investigation of ^{31}P-NMR relaxation features in water solution as a function of pH. The relaxation mechanism has been defined and contributions by paramagnetic impurities have been characetrized.

Complexes with the paramagnetic Gd(III) and Fe(III) ions have been studied by interpreting paramagnetic relaxation rates at different molar ratios, ionic strengths and pH. Consideration of Solomon-Bloembergen equations yielded all the relevant structural and kinetic parameters of metal binding equilibria.

The ionic interaction with lysine has been finally characterized thus obtaining a good piece of information on the biological activity of phytic acid.

^{1}H NMR Kinetic Study of DMSO[1] Exchange on Hexakis(DMSO)-vanadium(III) at Variable Temperature and Pressure

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Solvent exchange on $[V(DMSO)_6](CF_3SO_3)_3$ has been studied as a function of concentration, temperature (240 to 370 K) and pressure (up to 200 MPa, at several temperatures)[2] by ^{1}H-NMR spectroscopy at 200 and 400 MHz. In order to obtain the exchange rates k_{ex} we used two techniques: at low temperatures (240 to 267 K) we measured the isotopic equilibration of a solution prepared by mixing rapidly solutions of $[V(DMSO)_6](CF_3SO_3)_3$ and DMSO-d_6 in the diluent CD_3NO_2; at higher temperatures and for the variable pressure measurements we used the broadening of the proton signal of the bound DMSO to monitor the exchange.

The observed rate constants in CD_3NO_2 varied linearly with the concentration of free DMSO leading to a second order rate law.

The linewidth of the bound DMSO signal can, in the case of slow exchange, be expressed as a sum of a term due to transverse relaxation and a term due to chemical exchange. The opposite temperature dependence of the two contributions allows to separate them. The activation parameters and rates at 298 K are given in Table I.

The pressure dependence of the line width of the bound signal was observed at four different temperatures in order to separate pressure effects on transverse relaxation and chemical exchange. The parameters calculated are shown in Table I.

TABLE I Parameters as obtained by non-linear least squares fitting of the variable temperature and variable pressure data (in pure solvent).

		$V(DMSO)_6^{3+}$	$V(H_2O)_6^{3+}$ [3]
k_{ex}^{298}	$/s^{-1}$	13.1 ± 1.5	500
$\Delta H^{\ddagger}$	$/kJ\ mol^{-1}$	38.5 ± 1.6	49.4
$\Delta S^{\ddagger}$	$/J\ K^{-1}\ mol^{-1}$	-94.5 ± 4.7	-28
$\Delta V^{\ddagger}$	$/cm^3\ mol^{-1}$	-10.1 ± 0.6	-8.9
$1/T_{2m}^{298}$	$/s^{-1}$	194 ± 5.8	
E_m	$/kJ\ mol^{-1}$	9.3 ± 0.5	
$\Delta V^{\ddagger}_m$	$/cm^3\ mol^{-1}$	-2.1 ± 0.4	

The second order rate law observed in the diluent and the negative values found for the entropy of activation, $\Delta S^{\ddagger}$, and especially the volume of activation, $\Delta V^{\ddagger}$, are in accord with an associative activation mode for the exchange reaction mechanism.

[1] DMSO = dimethyl sulfoxide $(CH_3)_2SO$

[2] The high pressure NMR probe will also be described

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SPIN LATTICE RELAXATION RATES OF TUNNELING CD_3 GROUPS

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Expressions for the spin lattice relaxation time T_1 of tunneling CD_3 groups in potentials of three- and sixfold symmetry are derived and compared with classical results.
In order to compute the relevant transition propabilities, we treat the orientational degrees of freedom in a quantum mechanical way. From the spin- and the orientational wave functions, total wave functions obeying the Pauli-principle are constructed and used for the calculation of the quadrupolar matrix elements.
The symmetry of the potential determines the transformation properties of the quadrupolar Hamiltonian, responsible for relaxation. Consequently, the correlation functions have to reflect this symmetry and the corresponding spin lattice relaxation rates contain only angular dependent terms, which are invariant under the operations of the point group considered. This fact leads to remarkable differences between three- and sixfold potential-symmetry.
The angular dependence of the spin lattice relaxation rate for a sixfold potential depends only on the orientation of the symmetry axis of the CD_3 group relative to the main magnetic field. For a potential of threefold symmetry this angular dependence is in addition determined by the orientation of the triangle built from the methyl-deuterons relative to this field.
In the first case it is possible to assign a unique T_1 to each frequency in the spectrum, but not in the latter one. Thus, for threefold potentials T_1 is expected to be nonexponential. This result was also found by Torchia and Szabo [1] for classically reorienting CD_3 groups without exploiting the full symmetry of the problem. However, this nonexponentiality should be more pronounced for tunneling CD_3 groups, because the spectra are not axially symmetric [2].
In conclusion, it should be possible to distinguish three- and sixfold potentials by measuring T_1.
Experiments of this kind are carried out on toluene-d_3, where the methyl group is considered to reorient in a threefold potential.

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^{1}H- and ^{3}H-NMR Relaxation Investigations of Inhibitors Bound to Metalloendopeptidases

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A general approach is presented to extract conformational parameters of inhibitors at the binding sites of metalloendopeptidases. The approach relies on obtaining ^{2}H- and ^{3}H-labelled inhibitors from precursors with localized double bonds. Single-selective and double-selective ^{1}H- and ^{3}H-NMR relaxation rates investigations in the presence of the enzyme yield dipolar interaction energies of selected ^{1}H-^{1}H or ^{3}H-^{3}H spin pairs, wherefrom the desired information is gained. Such approach is exemplified by the Suc-Pro-Ala inhibitor bound to bacterial Collagenase.

DYNAMICS OF PHOSPHATE HEADGROUPS IN MODEL MEMBRANES

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Many biological events which occur at the membrane surface depend on the structure and dynamics of the membrane components. The ^{31}P nucleus, naturally abundant in phosphatidylcholine lipids, is a potential reporter of structural and dynamical changes occurring at the interface. Dimyristoylphosphatidylcholine (DMPC), dispensed in excess water, was used to model, in a first step, the biological membrane. Multipulse sequences have been applied to both, unoriented and macroscopically oriented membrane samples over a wide temperature range. Lineshapes and echo intensities have been recorded as functions of interpulse delay times, temperature and macroscopic orientation of bilayer normal and magnetic field. For a successful analysis, it was necessary to experimentally separate the contributions of dipolar coupling and of chemical shift anisotropy. The dipolar (^{1}H-^{31}P) contribution to the transverse spin relaxation (T_2) was eliminated by application of a spin-lock sequence to protons, thus allowing very efficient decoupling with low power radio frequency. In case of the longitudinal spin relaxation (T_1), the amount of dipolar coupling was evaluated by measuring the maximum Nuclear Overhauser Enhancement (NOE).

The experimental results show that the contribution of the ^{1}H-^{31}P dipolar coupling and chemical shift anisotropy are highly orientation dependent. In particular, no dipolar contribution is observed when the bilayer normal is oriented at the magic angle with respect to the magnetic field. Maximum NOE was found ~ 30°C below the main transition. Plotted against the temperature, the T_1 and T_2 values exhibit distinct minima in the fluid and gel phase, respectively. In addition, both relaxation times show a pronounced variation with orientation angle between bilayer normal and magnetic field.

The obtained lineshapes and relaxation rates are presently analyzed employing a comprehensive relaxation model, based on the stochastic Liouville equation. Inter- and intramolecular motions in an anisotropic medium are explicitly considered. Computer simulations of the various experiments provide the order structures and dominant motional modes of the phosphate headgroups in membranes.

Tyrosyl radical relaxation enhancement in ribonucleotide reductase

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Every living cell that has to produce DNA needs ribonucleotide reductase activity in order to form the deoxyribonucleotides necessary for the DNA synthesis. The type of ribonucleotide reductase enzyme that is iron dependent consists of two protein subunits, in *E. coli* called protein B1 and protein B2. Protein B2 is a peptide homodimer and contains iron in a particular type of cluster with two high spin ferric ions antiferromagnetically coupled by superexchange through an oxo–bridge. (This center is very similar to the iron cluster in hemerythrin, which has the capacity to bind molecular oxygen reversibly.) To be active, protein B2 also must have a particular tyrosine residue oxidized to a radical state. This tyrosyl radical is formed when a reduced iron cluster is oxidized by molecular oxygen. This suggests that the radical is close to the iron center. While the ESR of the unperturbed radical may be studied at temperatures below 30 K, progressively increasing relaxation enhancement and line broadening is observed at higher temperatures. This confirms that the iron center and the radical are fairly close in space and fits with magnetic dipolar interaction between the radical and the antiferromagnetic iron center. Data obtained for two different enzymes, i.e. from *E. coli* (protein B2) and from mammalian cells (protein M2), will be presented and discussed.

NUCLEAR SPIN RELAXATION IN THE ROTATING FRAME

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The utility of the rotating frame in the context of nuclear spin relaxation measurements and two- and three-dimensional NMR experiments is discussed and exemplified by experimental examples using peptides and proteins.

Chain dynamics and NMR relaxation in phospholipid membranes.

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Abstract.

Static and dynamic properties related to the internal configurational motions have been calculated for the alkyl chains of phospholipid molecules in a membrane environment in the liquid crystal phase. The calculations have been performed for the chain 1 of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC), a typical constituent of phospholipid membranes. Under the assumption of fixed bond lengths and bond angles, the internal dynamics of the chain is described in terms of 15 dihedral angles. The time evolution of the angular variables is assumed to be diffusional in character, and a master equation for transitions among the stable conformers is constructed from the energetics and hydrodynamics of the chain. This method is an extension to the time domain of the rotational isomeric state (RIS) approximation, which has been widely used to compute static properties of the chains. After calculation of the suitable correlation functions, effective rate constants relevant for spectroscopic and kinetic observables have been computed. The position dependence of the rate constants along the chain has been examined with special reference to understanding the effects resulting from cooperativity in the conformational transitions.

The theoretical predictions are useful to interpret quadrupole splittings in ^2NMR spectra and selective T_1, $T_{1\rho}$ and T_2 experimental data.

MOLECULAR DYNAMICS STUDIED BY MODERN ESR METHODS

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RELAXATION OF HIGH-SPIN NUCLEI IN ANISOTROPIC SYSTEMS

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A variety of diffusive processes in complex fluids, such as liquid crystals and biological materials, occur on a time scale of nanoseconds or longer. Detailed information about such motions is contained in the spectral density functions determining the satellite linewidths in NMR spectra of quadrupolar I>1 nuclei, e.g. ^{23}Na (I=3/2), ^{17}O (I=5/2), ^{43}Ca (I=7/2). However, the satellites are usually subject to severe inhomogeneity broadening, due to a small (and unavoidable) residual orientational disorder or to temperature gradients. This problem can be circumvented by performing a **two-dimensional quadrupolar echo (2D-QE) experiment**, wherein the satellite amplitude is Fourier transformed with respect to the pulse delay τ in the QE sequence $(\pi/2)_x$-τ-$(\pi/2)_y$-τ-acq. The resulting spectrum features a central line with the same width – but devoid of inhomogeneity broadening – as the satellite from which it was derived.

The basic 2-D QE experiment , which probes the evolution of dipolar single-quantum coherence (1QC), can be modified in several ways. If the initial $(\pi/2)_x$ pulse is replaced by a suitable excitation sequence, one can monitor, instead, the rank-(2I-1) 1QC and thus obtain a spectrum with fewer (or inverted) satellites. This experiment can be used to reduce spectral overlap (in the case of small splittings) and to separate signal contributions from isotropic and anisotropic parts of a heterogeneous sample (since even-rank multipole magnetization cannot be generated in isotropic systems). In another variation of the 2-D QE experiment, we selectively refocus multiple-quantum coherence of order 2I-1. This experiment yields a triplet spectrum with inverted satellites and enhanced splitting. (For I=5/2, one gets a -5:14:-5 triplet with a 6-fold increase of the splitting). The central line is homogeneous and yields the same linear combination of spectral densities (with maximal weight for j_0) as the outermost satellite in the coventional 1QC spectrum.

We have recently exploited the 2-D QE technique in a ^{23}Na NMR study of the counterions in the reversed hexagonal mesophase of the Aerosol OT-water-isooctane system. This phase, consisting of very long aqueous cylinders hexagonally embedded in the continuous oil medium, can be oriented in a magnetic field. From 2-D QE, 2-D spin echo and inversion recovery experiments at 2 different orientations, we could extract all the spectral densities that characterize the relaxation behaviour in a system of this symmetry. In particular, the spectral density function describing counterion diffusion around the cylindrical interface was obtained at 3 frequencies from data at a single magnetic field. By varying the cylinder radius, we could thus determine the lateral diffusion coefficient for sodium ions near the charged interface.

PROTEIN SIDE CHAINS AS IRON LIGANDS

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NMR STUDIES OF CALCIUM-ENTRY BLOCKERS. III. RELATIONSHIPS BETWEEN THE RECEPTORS FOR CALCIUM ANTAGONIST AND FOR ENDOGENOUS ADENOSINE

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Various physiological effects of adenosine seem to be mediated by membrane-bound receptors coupled in inhibitory or stimulatory manner to adenylate cyclase. However adenosine receptors coupled to other effector systems, such as ion channels, may exist as well. In fact, some of the actions of adenosine may not be mediated simply by effects on adenylate cyclase and (ii) in some systems (e.g. in nerve terminals) effects on adenylate cyclase are not likely to occurr.

The general inhibitory effect of adenosine receptor agonists on neurotransmitter release suggests that adenosine might be exerting its effect in neurons via inhibition of calcium influx. Similarly, in eliciting vasodilation, adenosine may act by interfering with calcium entry and availability by a direct modulation of voltage dependent fluxes.

At the ligand binding level calcium-entry blockers have specific effects on both adenosine receptors and uptake sites, thus suggesting that adenosine sites are selectively coupled with the calcium-antagonist sites.

Here we present some evidence that the endogenous mediator adenosine affects binding of calcium-entry blockers at the surface of neutrophils as detected by enhancement of selective proton relaxation rates.

NMR STUDIES OF CALCIUM-ENTRY BLOCKERS. I. CONFORMATION OF NIMODIPINE, DILTIAZEM AND VERAPAMIL IN POLAR AND APOLAR SOLVENTS

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In the last few years, the use of a group of drugs collectively referred to "calcium antagonists" or "calcium-entry blockers", has been receiving growing attention. One of the most striking aspects of these drugs is their chemical heterogeneity opposed to their common action on voltage-dependent calcium channels.

The concentration differential between outer and inner cellular compartments is maintained through regulation of calcium-entry to the cell and the sequestering of free calcium within the cell into sarcoplasmic reticulum and mitochondria, or by the binding of calcium to the inner plasma membrane. Extensive research has indicated that calcium-entry blockers principally interfere with the entry of calcium into cells through voltage-sensitive channels.

In order to ascertain whether calcium antagonists belonging to different chemical classes (nimodipine is a dihydropyridine, diltiazem is a benzothiazepine, verapamil is a diphenylalkylamine) share common conformational features in solution, ^{1}H- and ^{13}C-NMR relaxation data has been obtained in polar and apolar solvents. In any case absence of structuring was observed and all compounds were shown to be characterized by high degrees of conformational flexibility.

NMR STUDIES OF CALCIUM-ENTRY BLOCKERS. II. INTERACTION OF NIMODIPINE AND DILTIAZEM WITH POLYMORPHONUCLEAR LEUKOCYTES

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It is known that activation of polymorphonuclear leukocytes (PMN) induced by particulate or soluble agents initiates a cascade of biochemical events that are mediated by a rise of free intracellular calcium and by an increase of the membrane permeability to calcium. It has been in fact recognized that calcium-entry blocker drugs inhibit aggregation, enzyme release and superoxide generation from PMN stimulated by formyl-methionyl-leucyl-phenylalanine or A23187. These findings have supported the evidence that PMN represents a useful experimental tool for investigating definite cellular functions and identifying specific targets of pharmacological agents.

Here we present evidence that two calcium-entry blockers belonging to different chemical classes (nimodipine is a dihydropyridine whereas diltiazem is a benzothiazepine) are both specifically and aspecifically binding to the surface of PMN in a manner that involves aromatic moieties of both drugs. The evidence of aspecific binding, as detected by enhancement of selective proton NMR relaxation rates, can be considered as the first step in delineating the conformation of bound calcium-entry blockers, with the aim of characterizing the different receptors for the different chemical classes.

STRUCTURAL INFORMATIONS ON METALLOPROTEINS FROM ELECTRONIC SPIN-LATTICE RELAXATION EXPERIMENTS

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Electronic resonance studies of the spin-lattice relaxation have known an important development during the last ten years in the domain concerning the biological electron-transfer systems. This approach is used as a complement to the conventional EPR spectroscopy to bring informations on the vibrations in the protein medium and on structural parameters which characterize the active sites.

The main applications concern i) the identification and characterization of the different types of redox centers. In our recent work on center X in Photosystem I from plants it is seen how to take advantage of the differences in the relaxation properties of the acceptors of PSI to improve the accuracy of the measurement of the stoechiometry of these centers, ii) the determination of spin-hamiltonian parameters, such as fine structure constants or exchange integrals in multinuclear redox centers, which can be simply related in favorable cases to the temperature dependence of the spin-lattice relaxation time, iii) the determination of the relative position of the active sites. In this case informations are deduced from the variations of the relaxation properties which are induced by magnetic interactions with fast relaxing neighbouring centers.

Different examples concerning heme and non-heme iron proteins are given to illustrate these applications.

INFLUENCE OF INTERNAL MOTIONS ON THE STRUCTURES DETERMINED BY NOESY EXPERIMENTS.

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The dipolar cross relaxation rate (a_{ij}) between 2 spins is a linear combination of the Fourier transform of the autocorrelation functions $G_1(t) = \langle Y^2_1(0).Y^2_1(t)/r^3(0).r^3(t)\rangle$ where Y^2_1 and r are the spherical harmonics and the modulus of the vector joining i and j, respectively. Because of the internal dynamics of biological molecules in solution, Y^2_1 and r are fluctuating functions and are generally correlated.

In the homonuclear NOESY experiment used for conformational analysis, the interproton distances are obtained from the equality $a_{ij}/a_{kl} = (r_{kl}/r_{ij})^6$. This approach assumes that 1)- Y^2_1 and r are independant 2)- $1/\langle r\rangle^6 = \langle 1/r^3(0).r^3(t)\rangle$. It is obvious that in many cases such approximations lead to erroneous distances.

In order to investigate the effects of the internal dynamics on the distances deduced from NOESY, simulations of motions have been performed for spin systems submitted to restricted brownian motions. The equilibrium coordinates of each proton are fixed so that the equilibrium distances $\langle r_{ij}\rangle$ are known for every proton pair. Time dependant brownian fluctuations are simulated by MONTE CARLO techniques, and the autocorrelation functions are computed. After Fourier Transform, the different true a_{ij} for the spin system are obtained. One of the proton pairs with an imposed constant distance r_{ref} is taken as reference. The value $r'_{ij} = r_{ref}(a_{ref}/a_{ij})^{1/6}$ is computed for the other couples of protons. It represents the distance that a NOESY experiment would have given for such a spin system. r'_{ij} can therefore be compared to the actual $\langle r_{ij}\rangle$.

The simulations have been applied to the system of spins formed by aromatic protons of a hexanucleotide in the B-form. The influence of the amplitudes of the motions and of the internal rotational diffusion constants has been investigated. It was found that, except for small amplitude motions, the difference between r'_{ij} and $\langle r_{ij}\rangle$ can be very important, depending on motional parameters.

NMRD of Gd(III) Macrocycles

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$Gd(DTPA)^{2-}$ and the polyazamacrocyclic $Gd(DOTA)^{-}$ are paradigms of general purpose paramagnetic complexes useful for enhancing contrast in MRI. It is of interest to determine how one might modify the chemical structure of these chelates to improve their utility for MRI in specific circumstances. We describe the results obtained on polyazamethylene phosphonate complexes of Gd^{3+}, comparing their NMRD profiles with those of their carboxylate analogs and with $Gd(DTPA)^{2-}$. A new derivative of DOTA, DOTA-PA, with a single acetate carboxyl group functionalized into a propylamide moiety was also synthesized. The chelate $Gd(DOTA\text{-}PA)^{-}$ was studied, including its thermodynamic and kinetic stability, its NMRD curve and a comparison with the profiles for $Gd(DOTA)^{-}$ and $Gd(DTPA)^{2-}$. The curves were fitted to various relevant parameters and, in favorable cases,independent results were obtained using luminescence, ESR and gel filtration techniques.

Syntheses of Novel Covalently Linked Porphyrin Quinones - EPR and ENDOR Characterization -

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Biomimetic approaches to structure and function in photosynthesis principally involve synthesis and physical characterization of molecules designed to model energy and electron transfer processes occurring in natural reaction centers. These model compounds are preferentially composed of a porphyrin donor covalently linked via a spacer to a quinone acceptor.

In this paper we report on the syntheses of three new porphyrin quinones using different types of spacers. It is our aim to develop synthetic strategies of general applicability allowing ready modifications of the different fragments.

It is demonstrated that the interpretation of the hyperfine coupling constants - previously obtained from ENDOR measurements performed on the respective paramagnetic derivatives - using advanced MO calculations (RHF-INDO/SP) enables conclusions about geometrical arrangements and electronic structures of the model compounds. Knowledge of these properties is a prerequisite for a better understanding of the electron transfer processes occurring in these systems.

Duplex DNA Structures by Complete Relaxation Matrix NOESY-distance Restrained Molecular Dynamics. Two-spin or Not Two Spin – That is the Question!

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Until very recently interproton distances from NOESY experiments have been derived solely from the two-spin approximation method. Unfortunately, it has been shown that even at short mixing times there is a significant error in many of these distances. In order to avoid the approximation of the two-spin method we have written a complete relaxation matrix program (*MORASS*: *M*ultiple *O*verhauser *R*elaxation *A*nalysi*S* and *S*imulation) which employs a matrix eigenvalue/eigenvector solution to the Bloch equations. Distances (from the rate matrix) can be directly back-calculated from the experimental NOESY intensity matrix by merging of the theoretical and experimental intensity matrices. A new structure is then calculated from the MORASS-derived distances by NOESY-distance restrained, molecular dynamics (AMBER). The resulting partially refined structure is then used in MORASS to calculate an improved theoretical intensity matrix which is once again merged with the experimental matrix. We have calculated the structures of a number of duplex oligonucleotides including 12-mer d(CGCGAATTGCGC), extra-helical base 13-mer d(CGC*A*GAATTGCGC), and various base-pair mismatch 12-mers d(CGXGAATTGX'GC) (X, X' = G,A, T, C, dU) by this iterative refinement approach using combined MORASS and restrained molecular dynamics calculations.

It is now widely appreciated that duplex DNA can exist in a number of different conformations. Significant conformational differences can exist globally along the entire double helix, or as local conformational heterogeneity in the deoxyribose phosphate backbone in the form of sequence-specific variations. We compare the sequence-specific variations in these oligonucleotides as determined by both the two-spin approximation and the merged MORASS/AMBER approach of the duplexes. While the main features of the B DNA structure are observed in these duplexes, most significantly, a number of sequence-specific variations in the conformation of the oligonucleotides are reproduced by these NOESY-distance restrained calculations.

^{31}P chemical shift and ^{31}P-^{1}H coupling constant data also serves as an important probe of the conformation and dynamics of nucleic acids, particularly the deoxyribose phosphate backbone, which is not well defined by the ^{1}H/^{1}H NOESY data. A major limitation in the use of ^{31}P NMR has been the difficulty in assigning the signals. A 2D ^{31}P – ^{1}H pure absorption phase, constant time NMR approach is shown to provide a staightforward, convenient method for assigning the ^{31}P signals of even moderately sized oligonucleotide duplexes.

It has long been our hypothesis that the dispersion seen in the ^{31}P chemical shifts in oligonucleotides are attributable to sequence-specific changes in the deoxyribose phosphate backbone. This hypothesis is now tested by the new ^{31}P assignment methodologies and a ^{1}H/^{31}P 2-Dimensional J correlated experiment. J(H3'-P) coupling constants (and hence the ϵ (C4'-C3'-O3'-P) torsional angles) have been measured for ten different oligonucleotides at different temperatures. Correlations between these experimentally measured P–O and C–O torsional angles show that these results are consistent with the hypothesis that sequence-specific variations in ^{31}P chemical shifts are attributable to sequence-specific changes in the deoxyribose phosphate backbone. These backbone torsional angles constraints may also be incorporated into the MORASS/AMBER NOESY distance-restrained molecular dynamics calculations to accurately define both base-pair *and* backbone geometries.

TWO-DIMENSIONAL SOLID STATE ^{13}C EXCHANGE NMR

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Information about the dynamic evolution of systems undergoing molecular reorientation is more directly obtained from the mixing-time dependence of 2D NMR spectra rather than from conventional relaxation methods: a 2D NMR exchange spectrum is identical to a two-time distribution function, whereas their integrals represent the correlation functions accessible through relaxation data. However, the validity of this interpretation largely depends on the availability of purely absorptive spectra and methods are described to obtain pure absorption mode 2D spectra of static and of rotating samples (MAS).

2D exchange NMR studies on natural abundance ^{13}C are presented for polyoxymethylene (POM) and isotactic polypropylene (iPP), where large angle jumps in the crystalline domains were detected.

ESR STUDIES OF DEHYDROGENASES AND ATPases BY MEANS OF SPIN-LABELED COENZYMES AND SUBSTRATES

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ß-Hydroxyacyl-CoA dehydrogenase (BHDH) catalyzes the NAD^+-dependent oxidation of L-3-hydroxyacyl-CoA to the corresponding ketone in the ß-oxidation of fatty acids. The nucleotide moiety of CoA is not essential, shorter substrates are highly active. The X-ray structure clearly showed the NAD binding site but left the orientation of the substrate in its binding pocket ambiguous (J.J. Birktoft et al., 1987).

NAD derivatives spin-labeled at either N^6 or C8 of the adenine ring are highly active coenzymes (40-60 % V_{max}) and yield strongly immobilized ESR spectra in complexes with the enzyme. We have now prepared active spin-labeled substrate analogs of acetoacetyl-CoA comprising the complete CoA moiety or structural components. Again, strongly immobilized ESR spectra were observed. These spectra show dipolar splitting between spin-labeled substrates bound to adjacent sites in the dimeric enzyme and allow for elucidation of the substrate's orientation in the binding pocket.

Photoaffinity spin-labeled derivatives of NAD^+ and ATP as introduced by us proved to be useful probes for ESR investigations under conditions in which non-covalent interactions are too weak for motionally restricted species to be easily observed. 2-Azido-ATP and 8-azido-ATP have been successfully employed as photoaffinity reagents for various ATPases. Because of its anti conformation the 2-azido derivative is to be preferred. We have now synthesized a spin-labeled analog (SL-2-azido-ATP) with the label being attached to the ribose moiety.

Irradiation of SL-2-azido-ATP in complexes with either mitochondrial or bacterial F_1ATPase or Ca^{2+}ATPase from SR resulted in its covalent incorporation. In case of F_1ATPase from the thermophilic bacterium PS3 as many as four equivalents of SL-2-azido-ATP were covalently attached.

With Ca^{2+}ATPase two strongly immobilized spectral components could be discerned in the ESR spectra indicative of two distinct ATP binding sites. Similarly, different binding sites were revealed in case of F_1ATPases. These sites can be attributed to catalytic and non-catalytic sites. In addition, with non-covalently bonded SL-2-azido-ATP spin-spin interaction between nucleotides bound to adjacent sites were observed indicating distancies between these sites of about 12 A.

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Syntheses and EPR/ENDOR Studies of Novel Modified Flavin Radicals

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Flavoproteins form an important class of enzymes found in almost all biological redox reactions. Since they are known to mediate between one and two electron transfer reactions paramagnetic species are involved in some of the flavin activities.

In this paper we report on the syntheses of a variety of isotopically labelled flavins including lumiflavin, riboflavin, FMN, and FAD. The paramagnetic derivatives of these compounds were studied by means of EPR and ENDOR techniques in isotropic fluid solutions. Moreover, ENDOR spectra could be obtained in frozen solutions of these radicals embedded within the pocket of flavoproteins (D-amino acid oxidase, riboflavin binding protein). It is demonstrated that specific deuteration enables unambiguous assignments of ENDOR signals to molecular positions.

AN EVALUATION OF THE PROTON MAGNETIC RESONANCE TEST FOR CANCER

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The line width of the proton magnetic resonance spectrum (MRS) of the composite methylene and methyl resonances of plasma has been reported as a marker for the presence of malignancy. In this study, the contribution of very low density (VLDL), low density (LDL) and high density lipoproteins (HDL) to the MRS line width was determined. This was achieved by measuring the MRS linewidths for the plasma from patients with primary disorders of lipoprotein metabolism and from normal individuals. A negative correlation between plasma trigylceride levels and the average linewidth was observed and this was confirmed in normal plasma to which pure VLDL was added. Also, computer simulations were employed to demonstrate how the line width varies in such complex mixtures of lipoproteins. We demonstrate that the line width is governed by the relative contribution of VLDL and HDL to the composite line shape. This is particularly important when the shoulder from HDL line lies near the half-height of the VLDL line. A changes in VLDL/HJDL ratio occur in patients with malignancy, we propose that this is the basis of the narrowed MRS lines observed in the proposed test for malignancy. However, any individual with elevated VLDL will be false positive in this test.

^{13}C NMR Relaxation Time Studies of Cholesterol in Dimyristoylphosphatidylcholine Vesicles

(B:167)

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Cholesterol has been shown by various techniques to regulate the fluidity of membranes[1] and numerous studies have been performed on model membrane systems containing cholesterol. Dimyristoylphosphatidylcholine (DMPC) was chosen in this study as the main lipid component as DMPC has a P_{β_1} (rippled bilayer phase) to L_α (liquid crystal bilayer phase) phase transition of 23.8 °C[2] which is conveniently below the normal body temperature of 37 °C. The vesicle form was studied in order to achieve sufficient sensitivity to enable the accurate measurement of ^{13}C NMR relaxation parameters. All samples contained 100 mg of DMPC with 2.0 ml of TRIS-EDTA buffer and 0.5 ml of D_2O (as on NMR deuterium lock) and the required amount of cholesterol. The vesicles were created by sonication.

At a frequency of 30.56 MHz, ^{13}C NMR spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) measurements have been performed on [4 - ^{13}C] cholesterol and [3 - ^{13}C] cholesterol (97.9 atom % ^{13}C) in DMPC vesicles over the concentration range 0 to 30 mol % and over the temperature range 28 to 50 °C.

The T_2 values of [4 - ^{13}C] cholesterol in DMPC are consistent with those for [4 - ^{13}C] cholesterol in dipalmitoylphophatidylcholine (DPPC) vesicles previously reported at the same frequency.[3] For both the C_3 and C_4 carbon atoms, T_2 increases with increasing temperature but decreases with increasing cholesterol concentration. Within experimental error, T_2 is the same for both the C_3 and C_4 carbon atoms.

In contrast, for both the C_3 and C_4 carbon atoms, T_1 is observed, within experimental error, to be independent of cholesterol concentration and temperature. For the C_3 carbon atom, T_1 is 0.30 ± 0.02 s whereas for the C_4 carbon atom it is 0.16 ± 0.02 s. Thus, within experimental error, the T_1 for the C_4 carbon atom is twice that for the C_3 carbon atom. This is to be expected since the steroid ring of the cholesterol behaves as a rigid structure and the relaxation rate is, to a good approximation, proportional to the number of directly bonded H atoms adjacent to the carbon atom.[4]

The C_{21} methyl peak of cholesterol at 17.37 ppm with respect to Tetramethylsilane (TMS), was observed, even though the cholesterol was not enriched at this site. The associated value of T_2 was long, (approximately 0.1 s), as a consequence of the rapid reorientation of the methyl group.

In contrast to previous workers,[5] we have not detected a shift (deshielding) of the carbonyl resonance with increasing cholesterol content. This sheds doubt on the popular model in which there is hydrogen bonding between the OH group of cholesterol and the carbonyl groups of DMPC.

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ELECTRON SPIN ECHOES IN ZERO MAGNETIC FIELD

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EPR OF RADICAL PAIRS IN THE PRIMARY STEPS OF PHOTOSYNTHESIS

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Chemical reactions often result in electronically, vibrationally or rotationally excited molecules. It is less well known that chemical reactions in magnetic fields often produce paramagnetic species in non–equilibrium electron spin states, that is to say with spin alignments that differ from the Boltzmann distribution at the ambient temperature (1,2). These effects go by the name of chemically induced dynamic electron polarization (CIDEP) or, more simply, electron spin polarization (ESP). EPR intensities of polarized species may be enhanced by up to two orders of magnitude with individual resonances appearing in absorption or emission depending on the sense of the polarized population difference.

ESP of free radicals in liquids is well understood and is a sensitive probe of a variety of processes including photophysics and photochemistry, molecular motion, the interactions within and between molecules, chemical kinetics and electron spin relaxation. However, initial attempts to interpret the polarized EPR spectra of photosynthetic systems using existing theories of ESP were largely unsuccessful (3–5). In liquids, radicals formed in pairs normally separate rapidly so that EPR detects isolated, non–interacting free radicals. In photosynthetic organisms, by contrast, translational motion is restricted enabling EPR spectra of radical pairs with appreciable electron–electron exchange and/or dipolar interactions to be observed. Rapid formation of such radical pairs in spin–correlated states gives rise to strongly polarized EPR spectra (6,7). Similar effects have also been observed in viscous liquids and in micellar solutions where translational motion is hindered (8,9).

The theory of ESP in radical pairs and its origin in photosynthetic reactions will be outlined. The interpretation of these effects to give structural information on the primary steps of photosynthesis will be discussed.

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"Nitrosyl-hemoglobin: EPR- and ENDOR-studies of heme-globin interactions in single crystals and frozen solutions of myoglobin and hemoglobin and of hybrids".

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Nitrosyl-hemoglobin (HbNO) is a paramagnetic model for probing the molecular basis of the reversible, cooperative oxygen binding in hemoglobin. HbNO undergoes quaternary structural changes detectable by Electron-spin resonance (ESR) spectroscopy which can be related to the R <--> T transition in the quaternary switch of the oxygen affinity of hemoglobin. Consequently, the model has attracted considerable interest. Several ESR-studies over the past years have adressed some basic aspects like the stereochemistry of the NO-ligand binding and the spin-density distribution in the Fe-NO complex. We have applied, in addition, the electron-nuclear-double resonance (ENDOR) spectroscopy which expands, due to its increased spectral resolution, greatly the potential detail of information concerning the interaction of protons and nitrogens of the porphyrin core and of the proximal and distal amino acid residues with the Fe-NO complex. Combining single crystal ESR-ENDOR of myo- and hemoglobin, the isolated α- and β-subunits and ligand hybrids (αNOβX) and (αXβNO) in R- and T-states we can arrive at a fairly concise picture of the interplay between the heme-groups and the globin chains in both quaternary as well as in intermediate states. In this lecture we emphasize the relation between single-crystal and powder-ENDOR patterns, present powder-simulation routines and give a detailed account on hybrid hemoglobin in the frozen solution state.

ELECTRON PARAMAGNETIC ROTARY RESONANCE

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Numerous authors have discussed the influence on the transverse magnetization of two radiofrequency waves simultaneously incident on a single NMR transition. Chiarini *et al*[1] have considered the effect of two coherent resonant microwave frequencies on the z-component of magnetization in an EPR context. We have found no citations where the effects of two coherent resonant microwave frequencies on the transverse EPR magnetization have been considered. Nor do there appear to be reports where the displayed double resonance NMR signal was the output of a phase sensitive detector (PSD) operating at the difference of two coherent frequencies (or a harmonic of the difference). This experiment has been set up at X-band. A single sideband modulator (SSM) produces a 100 kHz sideband of comparable intensity to that of the carrier. Both sideband and carrier are incident on the sample. Detection is at the carrier frequency followed by a PSD at 100 kHz. Strong signals comparable to ordinary field modulation EPR spectra have been seen in numerous systems. Line shapes change from dispersion-like to absorption-like as the reference phase of the PSD is varied and also as the microwave reference phase is varied. Microwave power saturation is not a requirement for observation of the effect. Because pure absorption signals are detected with reasonably good baselines, this approach may be a practical alternative to field modulation.

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THE GIBBERELLIC ACID: A NUCLEAR MAGNETIC RELAXATION STUDY OF A PLANT HORMON.

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A full understanding of the role of plant hormons in determining biological events in plants is not obtained yet. Moreover it was not completely elucidated the main functional activity of the five plant hormon groups known, i.e. auxins, gibberellins, cytokinins, abscisic acid and ethylene.
The aim of this work is the analysis of the structure of a class of plant hormons: the gibberellins. Our attention was focused by the gibberellic acid (GA_3) one of the most rappresentative molecule of this class of hormons.
The elucidation of the structure of the gibberellic acid in solution was obtained by nuclear magnetic relaxation experiments. In particular selective relaxation techniques were used for the analysis of homonuclear dipolar connectivities (selective and biselective proton spin-lattice relaxation experiments) and carbon spin-lattice relaxation rates for the investigation of dynamical properties. On the basis of these experimental methods the accurate determination of selected i-j internuclear distances and hence the delineation of a preferred solution structure was posible. The combination of NMR experimental results and molecular mechanics calculation allowed a further refinement of the conformation of the Gibberillic Acid. This combined approach revealed great potential applications in the determination of detailed structures of biomolecules in solution.

Some aspects of biomolecular structure determination using 2D and 3D NMR

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For the structure determination of small biomolecules such as proteins and nucleic acids NMR spectroscopy now provides a realistic alternative to X-ray crystallography. NMR structures are primarily based on proton-proton distance constraints obtained from NOE's. Therefore, the range of structures and their accuracy depend critically on how many NOE's can be resolved and how accurate the distance constraints can be determined.

We have looked at both aspects of the structure determination by NMR. First, the resolution can be greatly improved by using 3D NMR methods. In particular, for the resonance assignment step, which is still the bottle-neck is studies of proteins, the non-selective ^{1}H 3D-NOE-HOHAHA experiment appears to be very useful. Assignment strategies based on this experiment will be discussed.

Secondly, the determination of distance constraints can be improved by taking into account the effects of indirect magnetization transfer (spin-diffusion) in the analysis of NOE's. For this purpose we have developed an iterative relaxation matrix approach (IRMA). The procedure has good convergence properties for both the distance constraints and the resulting structures.

It is also relatively straightforward to include dynamic processes such as aromatic ring flip and methyl group rotation in the IRMA procedure. Applications of these methods to DNA binding proteins (lac, lexA and Arc repressor) and protein-DNA complexes will be presented.

For invited lecture at: Third Chianti Workshop on Magnetic Resonance: Nuclear and Electron Relaxation, San Miniato (Pisa), Italy, May 28 - June 2, 1989.

COMPLETE RELAXATION MATRIX ANALYSIS OF 2D NOE SPECTRA FOR REFINEMENT OF PROTEIN AND DNA STRUCTURES IN SOLUTION D.J. Kerwood, M. Gochin, B.A. Borgias, and T.L. James, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 USA

A vast array of specialized 2D NMR experiments is now almost routinely used for spectrum assignment and structural characterization of nucleic acids and proteins. Here we will principally examine the use of the homonuclear 2D NOE experiment; it has potential for providing numerous interproton distances. Use of 2D NOE spectra for determination of modest-sized (≤20000 daltons) macromolecular structures is becoming widespread. While much success has been achieved using this technique to provide distance constraints for distance geometry or molecular dynamics calculations leading to structures, there are certain limitations and precautions that are not always appreciated. Refinement of protein structures via x-ray crystallography improved substantially with the development of programs for structure optimization directly against the primary diffraction data rather than against a derived quantity, the electron-density distribution. Likewise, it is possible to improve solution structures by refining against the 2D NOE spectral intensities directly.

Problems addressed with DNA and with protein structure studies are often of a different nature. Usually we are interested in fairly subtle structural changes in the DNA helix which are sequence-dependent. These subtle variations demand accurate distance determinations. But one can probably define a protein tertiary structure with moderate accuracy using distance geometry or restrained molecular dynamics calculations without accurately determining interproton distances; a qualitative assessment of distances is sufficient for calculation of a modestly high-resolution protein structure in solution. But, in proteins possessing less common structural features, it may be especially valuable to have more accurate interproton distances for use with the computational techniques. And, even more importantly, we will want better defined structures at ligand binding sites. Use of a complete relaxation matrix approach[1,2] (CORMA) to ascertain interproton distances from 2D NOE peak intensities offers the opportunity of determining protein solution structure with greater accuracy and resolution[3]. There are different methods of analyzing 2D NOE spectra for internuclear distance and structural information. The most effective techniques employ iterative refinement against experimental 2D NOE spectra, utilizing our program CORMA to calculate theoretical spectra of the molecular structures during refinement, in concert with molecular mechanics, molecular dynamics or distance geometry calculations. We have developed a program COMATOSE (*co*mplete *ma*trix *a*nalysis *t*orsion *o*ptimized *s*tructure) for *refinement* of structures by minimizing the error between the calculated and experimental 2D NOE spectra[3]. Experience with COMATOSE to refine experimental data has revealed that it leads to interproton distances in better agreement with experimental 2D NOE spectra than do the best "manually" manipulated models. As COMATOSE exhibits the familiar local minimum problem, we have subsequently written a program MARDIGRAS (*m*atrix *a*nalysis of *r*elaxation for *di*scerning *g*eometry of an *a*queous *s*tructure) which also iteratively fits theoretical and experimental 2D NOE spectra. Work so far indicates that MARDIGRAS is robust and displays negligible dependence on the initial model.

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ELECTRON SPIN-ECHO STUDIES OF STEARIC ACID NITROXIDE PROBES IN VESICLES AND MICELLES: RELATION TO INTERFACE HYDRATION AND ALKYL CHAIN CONFORMATIONS

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Electron spin-echo studies have been carried out for a series of doxylstearic acid spin probes (x-DSA where x = 5,7,10,12 and 16) in rapidly frozen vesicular and micellar solutions. Normalized deuterium modulation due to interactions of the unpaired electron on the nitroxide groups with deuteriums in water at the surfactant assembly interface or with deuteriums in specifically deuterated surfactants have been measured as a function of x.

In dodecylsulfate anionic micelles it is found that the counterion affects the conformation of the surfactant alkyl chains. With sodium counterion the surfactant chains are relatively extended but with lithium and tetramethylammonium counterions the surfactant chains are more disordered with some bent and U-shaped conformations. In nonionic micelles composed of polyethylene oxide surfactants the nitroxide probes are mainly located in the ethylene oxide region. The results show no water penetration into the alkyl core of the micelle and relatively little water penetration into the ethylene oxide region. It is estimated that only 1 - 2 ethylene oxide groups out of 6 - 8 are hydrated. In anionic micelles water also does not penetrate significantly into the alkyl region and the counterion controls the degree of water penetration into the headgroup region.

In vesicle systems dimethylammonium headgroups are more hydrated than choline headgroups when comparing zwitterionic phospholipid vesicles and cationic dioctadecyldimethylammonium chloride (DODAC) vesicles. The alkyl chains show a greater tendency for bending in DODAC vesicles compared to phospholipid vesicles. Addition of cholesterol to phospholipid vesicles increases the degree of interface hydration. This is analogous to the effect of n-butanol on sodium dodecylsulfate micelles.

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Reconstruction of the relaxation matrix and structure determination.

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Three major stumbling blocks are encountered during the structure determination of macromolecules by NMR:

- the spin diffusion
- the internal motion
- the fact that, due to overlaping, all Overhauser effects (NOE) between protons can not be quantitatively resolved, even in 2D experiments.

Not taking into account the two first problems leads to wrong calculated distances. This becomes really crucial when there is no folding of the molecular chain, which would provide constraining contacts between remote residues in the sequence. This is the case of nucleic acids, which represent a real challenge in the field of structure determination , or of small peptides. Here, the distances are to be estimated between protons of neighbouring residues. To be usefull, these distances must be calculated accurately.

To include all pathways of magnetisation tranfer, one should use the complete set of Bloch equations for the longitudinal magnetisation, which can be written under the following vectorial form:

$$dM / dt = - \Gamma (M-Mo) \qquad (1)$$

where M is the vector of all longitudinal magnetisation of all interacting protons, (M-Mo) is the vector formed by the differences between magnetisations at time t and at equilibrium, and Γ is the relaxation matrix:

$$\Gamma = \begin{bmatrix} \rho 1 & \sigma 12 & \sigma 13 & \dots & \dots \\ \sigma 21 & \rho 2 & \sigma 23 & \dots & \dots \\ \sigma 31 & \sigma 32 & \rho 3 & \dots & \dots \\ \dots & \dots & \dots & \dots & \dots \\ \dots & \dots & \dots & \dots & \dots \end{bmatrix}$$

Equation (1) directly transforms in:

$$dN / dt = -\Gamma N \qquad (2)$$

where N is the NOE matrix. Integrating the equation (2), in the case of 2D experiment gives:

$$(N) = \exp(-\Gamma.tm)(No) \qquad (3)$$

where tm is the mixing time and No is the NOE matrix for tm = 0.

This provides directly the relaxation matrix:

$$\Gamma = -(1 / tm)(\ln No^{-1}.N) \qquad (4)$$

This method in fact takes in account the spin diffusion, but it implies the knowledge of the full NOE matrix. This ideal case is hardly encountered for macromolecules.

We propose a method to reconstruct the relaxation matrix from an incomplete set of NOEs. The goal is to determine the relaxation matrix which minimise the difference (the X^2) between the calculated NOEs (using eq. 3) and their experimental values. The method is based on the fact that we know the analytical expression of the X^2 with respect to the relaxation rate constants. This allows us to apply a non linear least square minimisation procedure without resorting to any type of modelisation. At this stage, no hypothesis on the internal motion is necessary.

We have applied this method to the oligonucleotide $d(CGTACG)_2$. The cross relaxation rates obtained between protons separated by a fixed distance (H6-H5 of cytosines, H2'-H2" and H1'-H2" in sugars) give a direct measurement of the apparent correlation times in different part of the molecule. As expected, internal motion is observed in sugars. However, the correlation times observed for the motion of the H2'-H2" vectors vary all along the sequence, while those observed for H1'-H2" are constant and smaller than the former. These observations allow a modelisation of the internal motions, and the correct interpretation of other cross relaxation rates in term of averaged distances. The structure of the oligonucleotide derived from this analysis will be presented.

PROTEIN-PROTEIN INTERACTIONS, CONFORMATIONAL CHANGE, AND THE PHASE TRANSITION IN EYE LENS PROTEINS.

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Diamagnetic solute protein in water induces a magnetic field dependence of the magnetic relaxation rate $1/T_1$ of water protons, known as an NMRD profile. From analyses of the profiles of solutions of γ_{II}-crystallins (the smallest but best studied of the eye lens proteins) obtained from calf lens, we found that monomers oligomerize reversibly at relatively low concentrations: a ~30-fold increase in mean solute size is observed on going from 3% protein at 35°C to 16% protein at −4°C. At higher concentration, above ~16% by volume, much of the protein undergoes a change in tertiary conformation: the monomeric units reconfigure rather abruptly, increasing the interaction of protein NH groups with solvent, as evidenced by the onset of "^{14}N peaks" in the NMRD profiles. These arise from cross-relaxation between solvent protons and NH protons of crystallin amino acid residues. The peaks for γ_{II}-crystallin, comparable in magnitude to those reported previously for calf lens nuclear homogenates, are an order of magnitude greater than those observed in other tissues, and are not observed in solutions of other proteins, e.g. hemoglobin and myoglobin, at similar concentrations. In addition, judged from the variation of the monotonic part of the NMRD profiles, the tertiary structure appears to reorganize into an extended and open polymeric network, consistent with the appearance of NH peaks, and in a manner that may be unique to crystallins. We have followed these effects through the phase separation and find, as anticipated, few changes in the NMRD profiles: the method is insensitive to long range density fluctuations. Finally, we speculate on the evolutionary pressures that led to these properties.

2/20/89, For San Miniato, 5/89.

ESR study of hydration, unsaturation and curvature effects on slow tumbling dynamics of lipid bilayers.

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Abstract

The local anisotropy and molecular motion in lipid bilayers not only depend on intrinsic properties such as hydration, temperature or chemical composition, but on the geometrical packing configuration of the system as well. Consequently, the question of consistency of experimental results from different configurations has to be addressed.

We therefore compared ESR experiments on Cholestane spin labeled phospholipid systems in planar bilayer, liposome and vesicle configurations. The spectral simulations were based on the Stochastic Liouville Equation for slow molecular tumbling. A subtraction method considerably reduced the computational effort for the angle averaging required for vesicle and liposome spectra.

The various configurations are characterized by different macroscopic geometry, headgroup hydration and surface curvature. Taking these aspects into account, the experimental results are indeed mutually consistent, with the proviso that the planar bilayer configuration affords higher sensitivity with respect to details of the spectral lineshape.

While microscopic orientational order and tumbling motion are inversely correlated under hydration and temperature variations, changes in surface curvature and lipid unsaturation turn out to have clear effect on the orientational order only. This demonstrates that molecular order and reorientational dynamics are not necessarily correlated as is implied by the common concept of membrane fluidity. The work is currently being extended to lipid systems in hexagonal configurations.

Abstract for Chianti III

NMR RELAXATION AND MOLECULAR DYNAMICS SIMULATIONS FOR BINARY LIQUID SYSTEMS

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The dynamics of molecular motions in binary systems of small molecules in solution is investigated using the nuclear spin relaxation measurements and molecular dynamics simulations.

Results are presented for two types of systems. First, we consider solutions of quinuclidine. According to the NMR data, quinuclidine reorients isotropically in non–interacting media, while the rotation becomes strongly anisotropic in hydrogen–bonding solvents. Benzene holds an intermediate position. The large–scale molecular dynamics simulations were performed for quinuclidine solutions in benzene and in water. It is demonstrated how the interplay between experiments and simulations can provide a fairly detailed picture of the dynamic effects of intermolecular forces.

The second category of systems are mixtures of acetonitrile and chloroform. The experimentally observed variation of reorientational correlation times for the symmetry axes of the symmetric tops with the mixture composition can be explained in a qualitative way in terms of intermolecular forces in the system. The multinuclear relaxation studies also permit a critical evaluation of models for rotational diffusion. MD simulations are shown to be a valuable complement to the experiments.

ORIENTATION AND MOTION IN POLYMERIC MESOPHASES: NON-LINEAR ESR SPECTROSCOPY STUDIES L.Andreozzi, M.Giordano, D.Leporini, M.Martinelli, L.Pardi, Dipartimento di Fisica, P. Torricelli 2 I-56100 Pisa, ITALY

Recently, a growing interest has been devoted to polymers and polymeric mesophases. Both spatial cooperative order and molecular dynamics of this wide class of materials are currently investigated. However, the role of the usual ESR spectroscopy/ spin probe technique has been severely hindered by: i) a marked decrease of the spectral sensitivity to the details of the probe dynamics when the microviscosity falls in the typical range of polymers, ii) the inhomogeneous broadening of the lines, blurring the very contribution of interest originated by the motion of the probe. Multiple irradiation CW schemes sensitive to longitudinal relaxation processes more than to transverse ones have been designed by the authors in an attempt to overcome the above drawbacks. We have applied these techniques to study both static and dynamical properties of selected mesomorphic polymers. We present some novel results proving that longitudinal relaxation processes are effectively affected by microdynamics. We have observed meaningful variations of the linewidth at different temperatures. Furthermore, we have proven that the linewidth changes noticeably depending on the particular group of irradiated spin-packets. This feature suggests a useful display of the results via contour plots whose properties will be outlined.

CONFORMATIONAL PROPERTIES OF SOME SELECTIVE ß-ADRENERGIC DRUGS IN POLAR SOLVENTS AS OBTAINED BY ^{1}H- AND ^{13}C-NMR RELAXATION STUDIES

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It is known that the nature of the substituent at the amino group of α-hydroxyphenetylamines determines α- or ß-adrenergic activity, whereas the type and position of substituents at the aromatic ring determine the agonist or antagonist character of the drug.

With the aim of delineating the conformational features that trigger the ß-adrenergic activity, we have considered both isoproterenol and dichloroisoproterenol that are a ß-agonist and a ß-antagonist respectively and we have determined the conformational dynamics in polar solvents by thoroughly interpreting ^{1}H- and ^{13}C-NMR relaxation rates and scalar couplings. It has been apparent that the dichloro-derivative is characterized by a more pronounced degree of conformational flexibility.

Finally we have considered a class of recently synthesized drugs (N-isopropyl-N-[3-[4(4-alkyloxybenzoylamino)phenoxy]-2-hydroxypropyl] ammonium derivatives) that display strong $ß_1$-blocking adrenergic activity without appreciably affecting $ß_2$-adrenergic receptors. It has been shown that, in this case, extensive intramolecular H-bonding network yields a 'preferred' conformation in solution, thus preventing segmental motion along the quaternary ammonium sidechain.

Studies of the structure of cubic lyotropic liquid crystalline phases.

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Several lyotropic liquid crystalline phases with cubic symmetry have been observed both in surfactant/water and in membrane lipid/water systems. In phase diagrams determined for micelle-forming amphiphiles, as for example simple soaps, cubic phases can be found at different concentrations between any of the well-known micellar, normal hexagonal (H_I), lamellar, inverted hexagonal (H_{II}), and inverted micellar phases. For the swelling membrane lipids (generally not forming micelles) cubic phases may form at low water content and high temperature or may in some cases even be in equilibrium with a water solution. These lyotropic liquid crystals exhibit, like the lamellar and hexagonal phases, long range order but have disordered acyl chains. The aggregates formed by the amphiphilic molecules are located in a cubic three-dimensional lattice and quite many different cubic structures are known. Several physical methods are needed for an unambiguous determination of the various cubic phase structures and NMR has shown to be a very useful technique in many respects. In particular the NMR diffusion method is of great importance here but also the determination of T_1 and T_2 relaxation times and line shape analysis. Often also changes (studied by NMR) in the structure of the phases in equilibrium with the cubic phase can give an indication on the structure of the cubic phase.

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COMPUTER MODELLING OF THE STRUCTURE AND DYNAMICS OF LIQUID CRYSTALS

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The characterisation of structured fluids, such as liquid crystals, relies necessarily on the application of a wide range of techniques. However, it can be argued that NMR spectroscopy plays a particularly important role because of the wealth of information which it can provide. Thus for liquid crystals the transition frequencies are related to the long range orientational order while the spin relaxation times depend on both the order and the molecular dynamics [1]. In addition, because NMR is site specific, this information can be obtained for the numerous rigid sub-units which constitute the molecules of a typical liquid crystal. The many ingenious developments of the technique have also facilitated the experimental determination of such information, especially for quite complex molecular structures associated with liquid crystal formation.

An understanding of this detailed information, both static and dynamic, undoubtably benefits from a theoretical framework with which to describe the molecular behaviour of liquid crystals. The molecular field theory of nematics produced by Maier and Saupe and its evolution to mixtures, smectics and systems of biaxial as well as flexible molecules has been particularly important in understanding the orientational order in liquid crystals [2]. In addition the orientational dynamics, as described by the diffusion theory developed by Nordio and his colleagues has been invaluable in understanding a range of experimental results [2]. Despite the success of such analytic theories their applications are not without their problems, in particular the major approximations on which the theories are necessarily founded.

These difficulties can be overcome with the use of the Monte Carlo and molecular dynamics techniques of computer simulation, since these give essentially exact results for the behaviour of a collection of particles interacting with a given intermolecular potential [2]. The challenge in such an approach is to design pair potentials which mimic those of real liquid crystals and yet can still be investigated with the computational resources available. The first attempt to simulate the behaviour of a nematic was by Lebwohl and Lasher who employed a lattice model with particles interacting via a soft anisotropic potential [3]. Despite its simplicity this model was found to possess properties surprisingly close to those of real nematogens. Nonetheless the molecular interactions are far removed from those of real systems and in recent years more realistic potential models have been developed [4]. The most recent of these was proposed by Gay and Berne [5] based on their previous gaussian overlap model for rigid, cylindrically symmetric particles. The model has been studied using the molecular dynamics method [6] and found to possess three liquid crystal phases, nematic, smectic A and smectic B, as well as the isotropic liquid and crystalline phases. The occurrence of this relatively rich phase behaviour promises to make the Gay-Berne model mesogen especially valuable in understanding the properties of real liquid crystals. The structure of the phases are being characterised via orientational and translational order parameters as well as singlet distribution functions and pair correlation coefficients. In addition the molecular dynamics in the various phases are being determined via a selection of time dependent correlation functions.

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Contribution to the theory of CIDNP in viscous and micellar solutions

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CIDNP is considered to be a phenomenon that offers promising possibilities of insight into spin and molecular and chemical dynamics of radical pairs (RP). Former experiments and theories in this field usually refer to non-viscous solutions. In such systems, any anisotropic interactions are averaged to zero with a high efficiency such that spin dynamics are determined mostly by the isotropic part of Zeeman (isotropic g-values of radicals) and isotropic hyperfine interactions.

Properties of molecular dynamics, like reencounters of RP partners and characteristic life-time of RP's, turned out to be very important for CIDNP. Currently, there is much more interest rather to study viscous systems, systems with restricted diffusion of radicals like micellae etc.

We present some results of our theoretical analysis of how viscosity influences CIDNP, especially the magnetic field dependence of CICNP, and how anisotropic parts of Zeeman and hyperfine interactions can contribute to CIDNP in homogeneous and micellar solutions.

When the viscosity increases, then a maximum of the curve describing the CIDNP field dependence, can shift or the whole curve can become narrower. The contribution of anisotropic interactions to CIDNP depends on the degree of correlation of the thermal motion of the two partners in RP's. When their diffusion can be considered as being completely independent, one can interpret one effect of anisotropic interactions on CIDNP in terms of hyperfine component dependent rates of paramagnetic relaxation. This limit - relaxation mechanisms in CIDNP - was studied in more detail.

For the case of short-lived RP's, we generalized CIDNP phase rules (Kaptein rules) including anisotropic interactions alongside with isotropic ones. We also formulated some criteria for situations where isotropic or anisotropic contributions to CIDNP prevail.

DEUTERIUM NMR 2D EXCHANGE SPECTROSCOPY OF SOLUTES DISSOLVED IN LIQUID CRYSTALS

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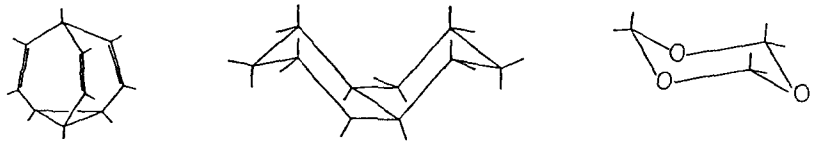
As in normal (isotropic) solvents it is also feasible to perform dynamic NMR studies of solute molecules dissolved in liquid crystalline (anisotropic) solvents. In certain cases liquid crystalline solvents may even be favorable to normal liquids, particularly when using deuterium NMR of deuterated solutes(1). We have recently extended these deuterium dynamic NMR studies to the 2D regime(2). Two dimensional exchange spectroscopy was first introduced by Jeener, Ernst and coworkers to spin I=1/2 nuclei in isotropic liquids(3) and subsequently by Spiess et al. to detuerium (I=1) in solids and polymers(4). Here we demonstrate the application of this method to several solute molecules dissolved in liquid crystals, including bullvalene(I), cis-decalin(II) and a number of saturated six-membered rings, e.g. s-trioxane(III).



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The main advantage of the 2D exchange method in liquid crystalline solution is in the extreme slow exchange limit where the reaction rate is too slow to affect the spectral lineshape. Under suitable experimental conditions the reaction rate and pathway, as well as the relative signs of the quadrupole interactions of the exchanging deuterons can be determined from the positions and intensities of the cross-peaks in the 2D-exchange spectra. In the case of *bullvalene*(5) a *succession* of cross-peaks appear as function of the mixing time according to whether they correspond to directly connected sites in the exchange scheme or indirectly via one or several intermediate steps.

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PROTON DETECTED ^{15}N NMR STUDIES OF LITTLE GASTRIN

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The key position occupied by the nitrogen atom in the peptide linkage makes it a potential source of NMR information on peptide secondary structure. The relatively recent development of Reverse Detection methods allowed the application of ^{15}N NMR to biological systems (1). The major difficulty in the reverse experiments is the suppression of large unwanted signals. Even if some methods have been so far suggested, water suppression in the reverse mode still lacks a general solution. We have compared different sequences and obtained better results employing the recently described sequence NEMESIS (2), a modified version of the reverse inept (1) in which the water suppression is accomplished with randomizing spin-lock pulses applied after the initial refocused inept sequence.

We applied these methods to 10 mM solutions of Nle^{15} little gastrin (pEGPWLEEEEEAYGWXDF-amide) at natural abundance ^{15}N. CD and NMR studies (3) in different solvents indicated that ordered structures are present only in TFE or in micelle solutions. We have identified the peptide ^{15}N resonances by correlating them with amide protons assigned through Tocsy and Roesy experiments. Measurements have been performed in different solvents and an analysis of the results is reported.

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VEGETATIVE TWO-IRON TWO-SULFUR FERREDOXIN I FROM *ANABAENA* 7120. SOLUTION STRUCTURE AND RECONSTITUTION STUDIES. John L. Markley, Byung-Ha Oh, and Lars Skjeldal. National Magnetic Resonance Facility at Madison, Biochemistry Department, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706, U.S.A.

A carbon-spin-system-directed approach (1) has led to assignments of nearly all 1-H, 13-C, and 15-N NMR signals in the diamagnetic spectral regions of oxidized ferredoxin I from *Anabaena* 7120. From these assignments, it is possible to deduce the sequence positions of residues affected by hyperfine interactions with the iron-sulfur cluster. The secondary structure has been determined on the basis of short-range NOEs, J-coupling, and hydrogen exchange patterns. A full three-dimensional analysis of the structure is in progress. The results to date indicate that this ferredoxin has a solution structure quite similar to the X-ray structure of the *Spirulina platensis* ferredoxin (2). Some minor differences found in secondary structural elements will be discussed.

Three methods for producing apoferredoxin appear to yield similar product: trichloroacetic acid, 0.1 M HCl, and 40 mM glycine at pH 3.1. The apoprotein is structureless by NMR criteria. In the absence of reductant (dithiothreitol), the cysteines of the apoferredoxin form disulfide bridges and are resistant to reaction with *p*-chloromercuribenzoate; this form of apoferredoxin also is structureless. Conditions have been found for reconstitution of the ferredoxin with iron and other metal ions. The absolute requirements for reconstitution and the refolding pathway are under investigation.

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Spin Label Relaxation and the Dynamics of Lipids and Proteins in Membranes.

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Conventional ESR spectroscopy of spin-labelled lipids and saturation transfer ESR spectroscopy of spin-labelled proteins have been used to study the rotational dynamics of the molecular components of biological membranes. 1) Simulation of the conventional ESR spectra from oriented dimyristoyl phosphatidylcholine bilayers containing spin-labelled lipids bearing a nitroxide free radical at one of three different positions in the sn-2 chain has allowed determination of the parameters governing the orientational, conformational and dynamic properties of the chain motion in lipid membranes. 2) Two-site exchange simulations of the conventional ESR spectra from spin-labelled lipids in reconstituted lipid-protein membranes have been used to measure the lipid exchange rates at the hydrophobic surface of integral membrane proteins. The intrinsic off-rates reflect the selectivity of the lipid-protein interaction and, in the case of the ADP-ATP carrier, give evidence for a slowly exchanging population of cardiolipin lipids associated with the protein. 3) Saturation transfer ESR has been used to study both the rotational diffusion and segmental mobility of spin-labelled integral proteins in membranes. A series of new spin labels with different modes of covalent attachment to the protein have been introduced to discriminate these two motions via the differential effects on lineshape and spectral intensity. The rotational diffusion coefficient reflects the aggregation state of the protein and has been used to study protein-protein interactions in reconstituted cytochrome oxidase membranes of varying lipid/protein ratio.

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Structure and clustering of VO^{2+} Ions in ionomeric Materials: ESR and ENDOR.

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Typical ionomeric materials are constituted by perfluorinated polymers consisting of organic backbone and ionic ($-SO_3^-$, COO^-) groups, and they are used in many chemical and electrochemical processes and devices[1]. The electron spin resonance spectroscopy of transition metal ions or of nitroxide radicals have been used to investigate both ionic mobility in ionic clusters and the unusual behavior of adsorbed water [2,3].
In this communication we report on an ESR and ENDOR study on Vanadyl aqueous solutions at different metal ion concentration adsorbed on the perfluorosolfonated membrane Nafion in its acidic form. The structure of this polymer has been the argument of several papers[4]. The analysis of the temperature dependence of the ESR parameters and of the ESr lineshape gave details on the structural and motional properties of both VO^{2+} and water molecules. The vanadyl probe maintained its square pyramidal symmetry. Adsorbed water was confirmed to maintain a relatively high mobility at temperatures significantly below 273 K. The occurrence in the ESR spectra of the typical half field transition ($\Delta m_s = 2$), usually observed for paramagnetic systems in a triplet state allowed us to establish that a small fraction of VO^{2+} ions aggregated in the ionomer as dimers with an intercationic distance $\geq$ 3.5-3.7 Å. Endor spectra gave VO-F distances of 10-13 Å which confirmed that the ions were prevalently located in ionic clusters that are formed after swelling with water.

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HUMAN SERUM ALBUMIN (HSA) IN REVERSE MICELLES STUDIED BY ESR

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A great number of proteins can be solubilized in hydrocarbon solvents by reverse micelles and small amounts of water (1). The proteins do not undergo, in many cases, relevant conformational changes and mantain their activity which sometimes, increases with respect to the aqueous solution.

We have carried out a study on HSA labelled with a nitroxide radical and solubilized in reverse micelles constituted by AOT (Bis(2-ethylhexyl)sodium sulfosuccinate) in isooctane.

The ESR spectra show relevant changes when the water amount added to the micelles is changed and indicate that the protein increases its mobility with increasing of the water content. At high values of water content the ESR spectrum does not change in a considerable way; however such a spectrum is different from the spectrum obtained in pure water, and we believe that some structural changes occur in HSA when hosted in reverse micelles.

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Sieroalbumina umana (HSA) in micelle inverse studiata tramite ESR

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E' stato dimostrato che un gran numero di proteine possono essere solubilizzate in solventi organici con l'aiuto di micelle inverse e piccole quantità di acqua (1). Tali proteine non subiscono, nella maggior parte dei casi, rilevanti cambiamenti conformazionali e mantengono la loro attività che, in alcuni casi, risulta aumentata (2).

Abbiamo svolto uno studio sulla HSA, marcata con un radicale nitrossido, contenuta in micelle inverse costituite da AOT (bis-2-etilesil solfosuccinato sodico) in isoottano.

Gli spettri ESR mostrano notevoli cambiamenti al variare della quantità di acqua aggiunta alla micella e indicano che, all'aumentare del contenuto d'acqua, la proteina aumenta la propria mobilità. Per valori elevati del contenuto d'acqua si arriva ad una situazione in cui lo spettro ESR non varia più notevolmente; tale spettro è differente da quello che si ottiene per l'HSA in acqua pura, e crediamo sia un effetto del particolare ambiente sentito dalla HSA nella micella.

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ANGULAR AND FREQUENCY DEPENDENT NMR RELAXATION STUDIES OF LIPID MEMBRANES

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NMR relaxation studies in one and two dimensions have been employed to evaluate the dynamic organization of biological model membranes [1]. Generally, variation of pulse sequence, pulse separation, magnetic field orientation and magnetic field strength provides the large number of independent relaxation experiments necessary for a proper molecular characterization of the systems. Analysis of these experiments is conveniently achieved by employing a density matrix treatment based on the stochastic Liouville equation. Arbitrary relaxation rates, including their angular and frequency dependencies, are considered.

The ^2H NMR studies described here provide new information about the molecular organization of biological membranes. There is clear evidence for an orientational order parameter of much less than unity in L_α phase [2] and two different order parameters in the P_β phase. From the dynamic point of view, the primary result is the detection and characterization of the dominant motions, such as overall reorientation, internal isomerization and collective order fluctuations. By employing multipulse dynamic NMR techniques, it is possible to follow these motions over an extremely wide dynamic range, extending from 10^{-11} s to 10^{-2} s [3]. T_{1Z} dispersion measurements, carried out over a frequency range of six orders of magnitude, clearly show that collective order fluctuations do not constitute a major relaxation mechanism in the MHz range [4]. Rather, isolated motions of individual molecules fully account for the observed T_{1Z} data. Collective order fluctuations are observed only at extremely low frequencies in the kHz regime by a characteristic $T_{1Z}(\omega_o) \sim \omega_o^{1}$ dispersion law, predicted for smectic type liquid crystals [5].

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RELAXATION, CROSS RELAXATION AND SPIN DIFFUSION IN MESOPHASES

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Usually the dense proton spin system of organic liquid crystal molecules does not seem to be an attractive candidate to perform mesophase NMR-experiments. However recent approaches combining high-resolution solid-state techniques with relaxation measurements can give new insight into internal and external relaxation processes. Many relaxation measurements of magnetic Zeeman and dipolar order on protons have contributed to the determination of structure and dynamics in liquid crystal phases. Despite the complex spin system with usually different inequivalent spins in these molecules, exponential relaxation has generally been reported. Few studies only deal with processes that do equilibrate magnetic order among these different spins.

NMR cross relaxation and spin diffusion phenomena in thermotropic liquid crystals are experimentally investigated using two-dimensional exchange spectroscopy techniques. Spectral spin diffusion of Zeeman and dipolar order between resolved proton lines is observed in nematic and smectic phases of thermotropic liquid crystals. Mechanism and rates are discussed in the framework of relaxation theory.

Internal equilibrium of the spin system is achieved on a time scale faster by a factor of ten than equilibrium with the lattice.

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STRUCTURE AND ELECTRON TRANSFER DYNAMICS OF PRIMARY REACTANTS IN PHOTOSYNTHESIS - AN ENDOR/TRIPLE AND MO STUDY

The X-ray structures of reaction center (RC) single crystals of the photosynthetic bacteria Rps. viridis and Rb. sphaeroides confirm the existence of bacteriochlorophyll (BChl) dimers which were postulated earlier to be the primary electron donors P960 and P870. Apart from the spatial structure of these dimers a knowledge of the electron density distribution in various electronic states is indispensable for an understanding of their functional properties.
For $P870^+$ and $P960^+$ the electron spin density distributions were obtained by ENDOR/TRIPLE via the hyperfine couplings. The comparison between the EPR/ENDOR data of $P870^+$ and $P960^+$ in RC's and of monomeric BChl a^+ and BChl b^+ shows that the primary donors are supermolecules with more or less asymmetric spin densitiy distributions over the dimer halves.
Theoretical spin and charge densities were calculated by an all-valence electron SCF MO method, RHF-INDO/SP, using coordinates from refined X-ray data including the amino acid residues of the protein environment of the pigments. These calculations yield asymmetry ratios similar to those observed. Consequences of the asymmetries in the charge distribution with respect to the observed unidirectionality of the electron transfer dynamics will be discussed.

SECONDARY STRUCTURE DETERMINATION OF SALMON CALCITONIN IN CRYOPROTECTIVE MIXTURES

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Salmon calcitonin (sCT) has been investigated by NMR at 500 MHz in 90% $DMSO_{d6}$-10% $^{1}H_2O$ (v/v) mixture at 278 K. All backbone and side-chain resonances of the hormone have been assigned using high-resolution phase sensitive two-dimensional techniques. Analysis of the type and magnitude of the observed sequential nuclear Overhauser effects, the NH-αCH spin-spin coupling constants, and the $^{1}H/^{2}H$ exchange kinetics measured in 80% $DMSO_{d6}$-20% $^{2}H_2O$ (v/v) at 278 K, enabled prediction of the secondary structure. Overall, an extended conformation is the dominant feature of the solution, but there are clear indications for a short double-stranded antiparallel β sheet in the central region comprising residues 12-18, connected by a three-residue hairpin loop formed by residues 14-16. Two tight turns, made by residues 6-9 and 25-28, were also identified, but no evidence was found for the presence of regular helical segment. The β sheet favors an amphipathic distribution of the residues, orienting the predominantly hydrophylic Ser^{13}, Glu^{15} and His^{17} side chains above the plane of the sheet, and the predominantly hydrophobic Leu^{12}, Gln^{14} and Leu^{16} below it. This is interpreted as the "seed" of the amphipathic α helix postulated to be responsible for the interaction of sCT with lipids, a situation reminiscent of the folding mechanism of signal peptides in the interaction with membranes.

^{2}H NMR RELAXATION STUDIES OF LIQUID CRYSTAL POLYMERS

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Dynamic solid state ^{2}H NMR techniques are employed to evaluate the molecular properties of semiflexible liquid crystalline polymers (LCPs) within their different phases [1]. Besides the application of conventional 1D relaxation studies we report on a promising extension of these ^{2}H NMR methods using 2D procedures [2]. They allow for the direct representation of angular dependent relaxation times (T_{1Z}, T_{2E}) which is of importance for a reliable molecular characterization of complex dynamical systems exhibiting a superposition of various motional modes. The advantages of these 2D methods are demonstrated and contrasted with experiments on simple model compounds.

The main interest of our NMR studies concerns the complex solid state of the LCPs displaying a typical semicrystalline behavior, known from conventional non-mesomorphic polymers. 2D NMR relaxation studies reveal two LCP components assigned to a glassy (frozen nematic) and a crystalline polymer fraction. Investigations on quenched and annealed samples demonstrate a strong dependence of the relative amount of these fractions and their particular molecular motional behaviour on the specific thermal history. It is shown that the glass transition is related to the freezing of the overall rotational motions, while local conformational motions (phenylene ringflips, conformational jumps) remain unaffected. Although in the crystalline fraction some local conformational motions (trans-gauche isomerizations) of flexible methylene spacer segments occur, the phenylene groups of the bulky mesogenic units are completely rigid. In addition, the state of molecular order can be described by a high degree of conformational, orientational and macro-order, in agreement with the unusual material properties (good mechanical stability, high tensile strengths) reported for this polymer class [3]. Finally, the presented ^{2}H NMR studies of the various specifically deuteriated LCP samples allow the proposal of a structural model of the frozen nematic and crystalline LCP fractions which is supported by recent X-ray investigations on these compounds.

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NMR STUDIES OF THE ACTIVE SITE OF THE ACETYLCHOLINE RECEPTOR

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Dipolar Relaxation by Surface Diffusion

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Two-dimensional translational diffusion causes interesting phenomena in NMR relaxation and the implications for intermolecular dipolar interactions of spins have been well studied for more than 25 years. The most interesting feature is that the spectral density shows a logarithmic divergence towards zero frequency. But this phenomenon is closely related to the infinity of the motion. It was shown that either a finite lifetime within the plane or a finite size of the plane itself is sufficient to obtain well-defined values of J(0).

The present work is aimed to study a similar case which is related to many problems of practical importance. Also, in this model one spin diffuses in an infinite plane but the other spin is at a certain distance outside of the plane either fixed or moving in a parallel plane. This is an interesting intermediate case between 2D and 3D: the motion is 2D but the directions of the internuclear vectors span one half of a sphere. Explicit expressions were derived for the spectral densities and the limiting cases are discussed. It is shown that the divergence is absent in this case. The major peculiarities that remain are a non-exponential correlation function (decaying according to a power law) and a slight asymmetry of $J(\omega)$ in a log J - log ω plot. The model can be used conveniently to derive diffusion coefficients from experimental NMR data of mobile surface layers or intercalation compounds.

NMR STUDIES OF STRUCTURE AND DYNAMICS OF BIO-ACTIVE PEPTIDES

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The mono-dimensional frequency domain of a standard nuclear magnetic resonance spectrum is a very rich source of structural information. The recent use of 2D and 3D NMR spectroscopies has greatly enhanced the possibility of measuring chemical shifts and scalar coupling constants of complex spin systems. However, the most powerful information for determining solution conformations and molecular motions can be obtained in the time dimension of the nuclear relaxation process provided that the latter is dominated by the intramolecular dipolar relaxation mechanism. Thus, nuclear Overhauser Effects (NOE) from 1D and 2D spectra, ^{13}C and ^{1}H spin-lattice relaxation rates are the main tools for determining internuclear distances and molecular dynamics.

The fact that biological activities of many proteins are related to limited regions of their structures suggests that high resolution NMR may give a fundamental contribution to the understanding, at the atomic level, of the recognition process involving active protein fragments. A detailed conformational investigation of Active Site Peptides (ASP) in solution can be performed for defining the steric aspects of the biological function and for obtaining the structural base for a rational design of new drugs and vaccines.

For these molecular systems the structural analysis of relaxation data is not always straightforward since multiple conformational equilibria and/or Brownian motions which are intermediate in the NMR time scale may yield undetectable NOEs.

Then, a general strategy has to be followed to select the most appropriate experimental conditions and relaxation techniques for the investigation of each molecular system. The observation of inter-residue dipolar connectivities from conventional 1D or 2D NOE experiments is diagnostic of a secondary structure which can be resolved by calculating internuclear distances from the measured Overhauser effects. The absence of the latter NOEs even in ROESY 2D spectra can be ascribed to the backbone flexibility. In this case, information on the molecular dynamics can be obtained by measuring the spin-lattice relaxation contribution of selected internuclear vectors along the backbone and side chains. Stabilization of the native structure, may be achieved by chemical modification of the peptide with a NMR driven molecular design.

Syntheses and ENDOR Investigations of Novel Semiquinone Anions

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Biomimetic approaches to structure and function in photosynthesis principally involve synthesis and physical characterization of molecules designed to model energy and electron transfer processes occurring in natural reaction centers. These model compounds are preferentially composed of a porphyrin donor covalently linked via a spacer to a quinone acceptor.

It is the aim of this paper to examine a variety of modified semiquinones in order to develop particularly suitable electron acceptors as regards efficiency of the electron transfer in correspondingly modified porphyrin quinones.

ENDOR and TRIPLE resonance measurements yielded sets of hyperfine coupling constants including their signs. From these data conclusions about conformational preferences, e.g. of the cyclohexyl substituent could be drawn.

New results on spin relaxation by order fluctuations in nematic, smectic and reentrant liquid crystals

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Measuring and analysing the longitudinal nuclear spin relaxation time T_1 over a sufficiently broad range of Larmor frequencies or Zeeman fields, i.e. the relaxation dispersion $T_1(\nu)$ or $T_1(B)$, has proved to be one of the most direct and reliable methods to distinguish different kinds of molecular reorientation mechanisms by NMR. The technique is particularly important in the case of liquid crystals, where one has to disentangle more complex anisotropical motional processes than in normal isotropic liquids. By means of fast field-cycling devices [1] it has been possible to study the relaxation dispersion of numerous liquid crystals between Larmor frequencies of a few Hertz and about 100 MHz for protons [2], and thus to detect the basic characteristic dispersion profiles of collective reorientations (order fluctuations), namely the square root law $T_{1OF} \sim \nu^{1/2}$ for nematic and the linear dependence $T_{1OF} \sim \nu^{1}$ for smectic mesophases [3,4]. As a rule, these contributions dominate the relaxation rate at rather low frequencies not accessible without field-cycling [1].

Recent more systematic measurements on various homologous series (azoxybenzenes, biphenyls, phenylcyclohexanes) and some special mesogens (polymeric, ferroelectric, reentrant, lyotropic) reveal effects not easily describable by existing theoretical models. E.g. the magnitude of the OF contribution is not in quantitative accordance with available data on viscoelastic constants, the dependence of the long cut-off mode on material properties is smaller than expected, the nitrogen nuclei cause a triple instead of a single level crossing resonance, and some results are hard to combine with measurements of the dipolar relaxation time T_{1d}. These phenomena will be discussed in the lecture.

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The Effects of a Leader Sequence on Protein Structure and Dynamics

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Many proteins are made with a 20-30 residue leader sequence attached to the N-terminus that is subsequently removed. This leader sequence must have a large effect on the protein, since it is responsible for the insertion into or transport through membranes of the protein.

We are studying the influences of the leader sequence by comparing the structure and dynamics of a protein with and without the leader sequence. Procoat protein of the filamentous bacteriophage M13 has 73 residues, 23 of which are removed from the N-terminus, to form the coat protein. Samples of procoat and coat proteins labelled at various sites with stable isotopes have been prepared. The results of NMR experiments on these proteins will be described.

A SIMULATION BASED MODEL OF NMR T_1 RELAXATION IN LIPID BILAYER VESICLES

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The results of the Brownian dynamics simulation of a hydrocarbon chain in the mean field of a membrane bilayer are used to analyze the ^{13}C NMR T_1 relaxation in lipid bilayer vesicles. The analysis shows that the frequency dependence of the relaxation does not arise from gauche-trans isomerization or from axial rotation of the entire lipid molecule. However, a model in which fast axial rotation ($D_{\parallel} \approx 2 \times 10^{10}\ s^{-1}$) and slow non-collective diffusive director fluctuations ($D_{\perp} \approx 1\text{-}2 \times 10^{8}\ s^{-1}$) are superimposed onto the internal motions quantitatively accounts for both the magnitude and frequency dependence of the T_1 data. An effective viscosity for the interior of the bilayer in the range of 1 cp, and a director order parameter of 0.5-0.7 are required to fit the NMR data.

Relaxation of segment orientation in diluite polymer solutions. Interpretation of T_1 and NOE experiments on diluite poly-3,7-dimethyl-1-octene and poly-3-methyl-1-octene.

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The spin relaxation times of nuclei on a polymer chain in solution are sensitive to the chain segmental dynamics in the nanosecond time domain. Spin-lattice and spin-spin relaxation times T_1 and T_2 and the Nuclear Overhauser Effect, NOE, of ^{13}C over a range of resonance frequencies are the important NMR measurements to probe this segmental dynamics. The information on the dynamics is carried on the spectral density which is the Fourier transform of the second order correlation function (TCF2) of the relaxing dipoles. The crucial point for a quantitative interpretation of the time relaxation experiments by NMR is the availability of a confident dependence of the TCF2 on the local conformational details of the chain.

Here we try to give structral interpretation of T_1 and NOE experiments on poly-3,7-dimethyl-1-octene and poly-3,7-methyl-1-octene in terms of the stiffness of the chain and of the side chain correlation. To this aim the experimental data are analyzed using: (i) a TCF2 suitable for rigid backbone polymers(1); (ii) the TCF2 by Hall and Helfand, suitable for flexible backbone polymers(2); (iii) the TCF2 recently proposed by Perico and Guenza(3) for both flexible and semi-flexible backbone polymers. This last approach is based on the optimum Rouse-Zimm approximation to the generalized diffusion equation in the full polymer configuration space.

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AN NMR STRUCTURAL STUDY OF RAT EGF

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2D NMR studies were performed on rat Epidermal Growth Factor (rEGF), a hormonal polypeptide of 48 amino acids.

197 of the 200 non-exchangeable proton resonances were assigned, as were all backbone amide protons except for Asn1, and the side chain amide and amine protons of Asn16 and Arg41.

326 NOE's involving one or more exchangeable protons were seen, and 504 NOE's involving non-exchangeable protons. About 600 NOE's could be unambiguously assigned, although no stereo-specific assignments for methylene and methyl groups were obtained. From these data, 327 distance constraints could be derived. Using the distance geometry program DG, 19 structures were generated. For 10 structures, the allowed conformational space was further explored using Distance Bound Driven Dynamics (DDD). The maximum violations ranged from 0.07 to 0.7 Å in different structures.

The structures are distributed over two types of conformation. Group A resembles the published structures for human and mouse EGF. Group B structures resemble globally their mirror images.

Within a group, the rms differences between the coordinates is about 3.5 Å . The two types of structures fulfil the NMR constraints equally well. When analyzing all short proton-proton distances and backbone dihedral angles, it appears that there are very few significant differences between the two groups of structures.

More constraints are needed to decide which structure is correct.

MAGNETIC RESONANCE INVESTIGATION OF BIS-THIOSEMICARBAZONATO CU(II) COMPLEXES IN LIQUID PHASE

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It has been reported that ESR spectra for CuKTS vary as a function of pH between 7.5 and 1.0, although Copper remains tightly bound over this pH range. Computer aided ESR spectroscopy allows definition of equilibria between different species involved in solution. A multifrequency ESR approach combined with computer simulation of experimental spectra permits precise evaluation of rotational correlation time. An independent check of structural and dynamical configuration of the predominant species can be obtained taking advantage of ^{13}C and ^{1}H spin lattice and spin-spin relaxation rates by FT-NMR.

Evaluation of ion-proton distances can be interpreted in terms of conformational changes in the metal complexation for different antitumor agents. The precise measurement of τ_c by ESR spectroscopy can be utilized in the Solomon-Bloembergen-Morgan equations in order to precisely evaluate the number of coordinated ligands.

Analysis of the Transverse Relaxation Profiles of Methylene Protons in Magnetic Resonance Spectra of Human Tumours

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The transverse relaxation times of methylene proton resonances of lipids in magnetic resonance spectra of human tumours have been analyzed assuming a continuous distribution of relaxation times. We have studied 89 colon tumours (27 metastasized), 12 "normal" colon tissues, 40 breast tumours (24 metastasized) and 13 malignant lymph nodes. All tumour samples were primary tumours and the patients had received no previous treatment.

NMR experiments were performed on Bruker CXP 300 and AM 360 spectrometers at 37° C. The CPMG pulse sequence with a 1 msec delay was used to obtain transverse relaxation profiles of the prominent methylene peak at 1.3 ppm. The total delay time before acquisition ranged from 8 to 1348 msec.

In tumours, the decay of transverse magnetization of the methylene resonances is not a single exponential, whereas simple exponential decay is often observed for "normal" colon tissues (tissue from the pre-resection margin). The sum-of-exponentials approximation often leads to several solutions which fit the experimental decay; this is clearly a naive solution at best, given the complex mixture of resonances comprising this peak.

Two continuous distribution approximations were tested: the two-parameter lognormal distribution provides a simple representation of the relaxation profile. The constrained regularization method (Contin) can incorporate prior knowledge as constraints and provides a smooth, regularized and parsimonious relaxation profile consistent with noisy data. The average T_2 values, derived from the lognormal and Contin approximations are mutually consistent.

Tumour and "normal" colon tissue taken from the same patient show important differences in relaxation behaviour. A considerable broadening of the lognormal distribution, with $\langle T_2 \rangle$ shifted to shorter values, is observed for the colon tumours. This is verified by the constrained regularization method. The "normal" colon tissues are usually characterized by a single, relatively narrow distribution, while tumours show one or more broad peaks.

The different distributions of T_2 in cancerous and "normal" tissue should be useful for improving contrast in magnetic resonance imaging of patients suspected of having cancer.

Molecular motion in nematics EBBA and HBT studied with ^{13}C spin lattice relaxation

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The spin lattice relaxation of the orthoaromatic carbons in the nematic phase of EBBA has been measured at a Larmor frequency of 50 MHz. The single particle reorientational dynamics was assumed to be the only motion responsible for this relaxation (the frequency independence of these relaxation times supports this assumption) and the biphenylic core of the molecule was supposed rigid.

A small step rotational diffusion model was adopted for the description of the reorientational dynamics. Unfortunately in EBBA the presence of a single family of equivalent carbons does not allow the determination of the two independent diffusion coefficients $D_{\|}$ and $D_{\perp}$ and the latter has been drawn from fluorescence depolarization measurements [1]. Through the numerical solution of the diffusion equation and the adoption of an iterative procedure the diffusion coefficient relative to the spinning motion ($D_{\|}$) as well as the Overhauser enhancement factor (NOE), which allowed the separation of the pure dipolar part of the relaxation, have been determined.

The $D_{\|}$ values range, in the whole nematic phase, from $1 \cdot 10^9$ sec^{-1} to $5 \cdot 10^9$ sec^{-1} and, unlike $D_{\perp}$, exhibit an Arrhenius behavior.

The same measurements have been performed in the nematic phase of HBT (N-(4-hexoxybenzylidene)4'-toluidine) in which the presence of a methyl group directly attached to the biphenylic core of the molecule allows the determination of both the dynamical parameters.

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ADSORPTION OF NITROXIDE SOLUTIONS ON ZEOLITES; A STUDY OF THE ELECTRON SPIN ECHO DECAY FUNCTIONS

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The amplitude of electron spin echo (ESE) signals exhibits a decaying behaviour when registered as a function of the interpulse time.The analysis of this spectral feature can allow to get information on the relaxation mechanism affecting the spin system. We investigated the decay functions of two- and three pulse ESE patterns of nitroxide radicals in aqueous and alcoholic deuteriated solutions adsorbed on zeolites. The analysis was carried out by simulation of experimental spectra. Both the Gauss-Markof and sudden-jump models for the reorientation of the spins causing fluctuating magnetic fields (B-spins) were used to calculate the decay functions; the presence of nuclear modulation was also taken into account in simulations. To satisfactory reproduce the experimental results it was necessary to include into the decay functions a contribution due to the instantaneous diffusion caused by the flips of spins contributing to the echo (A-spins) induced by the pulses. The combined analysis of two- and three pulse experiments allowed to reach conclusions about theconcentration of adsorbed radicals. Differences were found to exhist depending both on the kind of zeolite and on the type of solvent. A discussion of these effects is also presented.

SOLUTION STRUCTURES OF RIBONUCLEASE T_1, ITS COMPLEXES WITH NUCLEOTIDES, AND RIBONUCLEASE T_1 MUTANTS

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The solution structures of Ribonuclease T_1 and its complexes with 2'-GMP and 3'-GMP have been investigated by combined use of 2D-NMR spectroscopy and restrained molecular dynamics (MD) calculations. The secondary structure elements of RNase T_1 were derived from the characteristic medium- and long-range NOEs. For RNase T_1, two antiparallel ß-sheets and one α-helix as well as some ß-turns were observed. The first ß-sheet is located near the N-terminus and involves two strands. The second ß-sheet is built up by five strands and forms most of the hydrophobic core of the protein. The α-helix of RNase T_1 is located between S13 and D29 and contains 4.5 turns. A low-resolution tertiary structure can be derived from corresponding long-range NOE data. The strands of the N-terminal ß-sheet are connected by a ß-turn. This ß-sheet is in contact with the N-terminal end of the α-helix. The α-helix is near the second strand of the large ß-sheet. Some contacts of the α-helix with side chains of the third strand are observed. The α-helix is followed by a loop. Extended loops are formed between strands 1 and 2 as well as between strands 2 and 3 of the large ß-sheet. Strands 3 and 4 of this sheet are connected by a ß-turn, and there is another loop between strands 4 and 5. The NOEs between residues Y4 to C6 and C103 to T104 are proof of the proximity of the N-terminal and the C-terminal ends formed by a disulfide bridge between C6 and C103.

The structural differences between free RNase T_1 and its 2'-GMP and 3'-GMP complexes are only small. Nearly identical NOE values were obtained for the free enzyme and its nucleotide complexes. Indications of structural changes induced by the complex formation can be obtained by comparing the chemical shifts of the resonances of the backbone protons in the free enzyme and those in its nucleotide complexes. The essential results may be summarized as follows:

1. Interaction of the inhibitors with the active site leads to changes in the conformation of the backbone of the active-site amino acids.
2. Conformational changes are also observed in the loop regions that are directly attached to the active site, and in the contact area between the α-helix and the five-stranded ß-sheet.
3. The structure of the RNase T_1-3'-GMP complex is more similar to that of the free enzyme than the structure of the RNase T_1-2'-GMP complex.

Restrained MD calculations were performed for the free enzyme as well as for the nucleotide complexes for 20 - 30 picoseconds in vacuo. About 600 NOE restraints were used in these calculations. The complexes between the inhibitors and RNase T_1 lead to a possible mechanism of RNase T_1-catalyzed RNA hydrolysis.

ELECTRON SPIN RELAXATION OF SPIN PROBES IN SOL-GEL GLASSES

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Electron paramagnetic resonance (EPR) spectra of nitroxide spin probes incorporated in silica sol-gel glasses were observed in the temperature range 4.5-300 K. The nitroxide probes used varied in size and shape. By varying process parameters, sol-gel glasses were obtained with different structures and pore sizes. EPR lineshape analysis was used to characterize the molecular motion of the paramagnetic spin probes located in the pores.

NEW EPR METHODS FOR THE STRUCTURAL DETERMINATION OF PARAMAGNETIC SPECIES

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Novel EPR techniques in both the frequency and the time domain with an improved structural information content compared to conventional cw-EPR are reviewed.

The survey includes

- Discrete saturation
- EPR-detected nuclear transient nutation
- Optimized pulsed ENDOR schemes
- New electron spin echo envelope modulation sequences
- Coherence transfer ELDOR

NMR DETERMINATION OF VISCO-ELASTIC PROPERTIES OF NEMATIC SIDE GROUP POLYMERS

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A sample holder containing a nematic side group polymer is rotated about an axis perpendicular to a magnetic field. The rotation speed of the container is chosen larger than the critical angular velocity $\omega_c = \chi_a B^2/2\gamma_1$, in which χ_a is the anisotropy of the magnetic susceptibility and γ_1 the rotational viscosity. Under this condition the director orientation follows the orientation of the container, allbeit with a lower mean angular velocity. We have synchronized the data acquisition with the container orientation. This enables us to record spectra of the deuterated side group as a function of container orientation. In order to follow the phase lag between container- and director orientation, the deuteron line splitting is used to monitor the director orientation. A simple relation holds between γ_1 and the phase lag. By this method extremely high values of γ_1 can be determined[1].

After a number of rotations the lines in the deuterium spectrum start to broaden until finally a steady state is reached. We discuss the possibility that this broadening is caused by backflow effects. When nematics with a large anisotropy in the viscosity are placed in a magnetic field perpendicular to the director orientation, periodic instabilities are being build up. These instabilities are produced by amplification of the amplitudes of the long wave length orientational fluctuations. A possible explanation for the occurrence of the steady state is discussed in terms of "frozen in" orientational waves i.e. the amplification is damped by the rotation.

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Applications of NMR in Studies of Enzyme Mechanism

by A. I. Scott

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Abstract

Site specific mutagenesis of Porphobilinogen deaminase, and the development of genetically engineered strains of *E. coli* has permitted the labeling of the enzyme with ^{13}C at the active site. ^{1}H and ^{13}C NMR experiments will be described which have led to a dynamic view of the mechanism of this ubiquitous enzyme, which mediates the assembly of natural type III porphyrins in bacteria, plants and mammals via a discernible cascade of successive covalent intermediates. The results will also be discussed in terms of the evolution of the biosynthetic pathway to Vitamin B_{12}, which in spite of many complexities, can also be unravelled by NMR spectroscopy.

ANIONS BINDING TO SUPEROXIDE DISMUTASE

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Bovine erythrocyte superoxide dismutase is a dimeric enzyme with each subunit containing a catalytically essential copper ion bridged by an histidinato residue to a solvent inaccessible zinc ion. The zinc ion can be replaced by cobalt without altering the catalytic properties of the enzyme. Such substitution gives rise to a strong magnetic exchange coupling interaction between the high spin cobalt(II) and the copper(II), characterized by an isotropic exchange coupling constant of 16.5 cm^{-1}.This interaction shortens the electronic relaxation time of the copper allowing to observe the ^{1}H NMR signals of the protons of the histidines coordinated to both the cobalt(II) and the copper(II) metal ions. In this way it has been possible to study the interactions between this derivative and anions that are known to be inhibitors of the enzyme. In particular azide, cyanate and thyocyanate have been reported to push His 44 away from copper coordination and this interaction has been suggested to be a general mode for the binding of singly charged anions, including the substrate O_2^-, to the active site of the enzyme. In this work we examined the behaviour of several anions and we propose the possible way of interaction between different classes of anions and the enzyme.

2D-NMR METHODS FOR STUDYING MOLECULAR MOTIONS

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Two-dimensional NMR offers unique possibilities for a detailed characterization of molecular order and dynamics in solids and solid polymers. This holds in particular for ultraslow dynamic processes occuring on a time-scale above 1 ms.

2D exchange spectra of static samples display characteristic ridge patterns, if the reorientational motion occurs around well-defined angles, e.g. in crystalline polymers. In amorphous polymers, on the other hand, rotational motions of both polymer chains and side groups typically involve rotations about ill-defined angles, leading to angular distributions. These are directly projected into broad exchange patterns of the 2D NMR spectra.

The angular resolution is highest for static samples, e.g. for ^{2}H-NMR of isotopically labelled samples or ^{13}C-NMR of simple polymers. The spectral resolution can be increased at the expense of the angular resolution ($\pm$ 10° instead of $\pm$ 1°) by invoking magic angle spinning (MAS). Rotor-synchronized MAS together with rotations in spin space, e.g. by the TOSS-sequence, then not only allows the detection of molecular order and dynamics separately but also offers a means to correlate these two molecular parameters, which are often supposed to govern the macroscopic properties of solid polymers.

The techniques will be described and demonstrated by studies of the chain motion in semicrystalline polymers with helical structure, amorphous polymers both below and above their glass transition and partially ordered liquid crystalline polymers.

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NUCLEAR SPIN RELAXATION OF ^{119}Sn AND ^{207}Pb IN METAL(II) AND METAL(IV) COMPOUNDS

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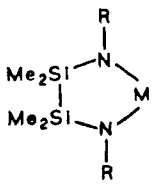
For normal diamagnetic solutions (motional narrowing limit $\omega_0^2\tau_0^2 \ll 1$) intramolecular interactions will be the dominant source for nuclear relaxation. Regarding half-spin nuclei, four different mechanisms have to be considered contributing to the longitudinal relaxation rate $(T_1)^{-1}$:

$$(T_1)^{-1} = (T_1^{DD})^{-1} + (T_1^{SR})^{-1} + (T_1^{CSA})^{-1} + (T_1^{SC})^{-1}$$

For the transverse relaxation rate $(T_2)^{-1}$ it holds that $T_2^{DD} = T_1^{DD}$, $T_2^{SR} = T_1^{SR}$, $T_2^{CSA} = 6/7\ T_1^{CSA}$ and $T_2^{SC} \ll T_1^{SC}$ owing to scalar interactions with quadrupolar nuclei (e.g. ^{14}N).

For the molecules **1** the chemical shift anisotropy (T_1^{CSA}, T_2^{CSA}) and scalar relaxation of the second kind (T_2^{SC}) are dominant. The contribution of T_2^{SC} to the linewidth of the metal NMR spectra can be calculated from the known magnitude of $^1J(M^{15}N)$ and the ^{14}N quadrupolar relaxation rate. The importance of the CSA-mechanism is shown by measurements at different field strengths as well as at different temperatures. Since the temperature dependence of these two mechanisms are opposite the metal NMR signal will be broad both at high and low temperatures. The short relaxation time of the metal nuclei M may also hamper the observation of M-satellites in X-NMR spectra (X = ^1H, ^{13}C, ^{15}N, ^{29}Si) for the determination of $^nJ(MX)$.



1

M = Sn, Pb
R = ^{t}Bu

Cross Terms between Dipolar and CSA Interaction.

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It will be demonstrated that the chemical shift anisotropy (CSA) relaxation for protonated carbonyl 13 C nuclei is by no means negligible. The cross correlation spectral density arising from dipolar and CSA interactions in the natural abundance 13C-1H spin system of dimethylformamide is included in the complete determination of molecular motional anisotropy.

In the following discussion we will use the normal mode analysis of relaxation data reviewed by Werbelow and Grant. Thereby inclusion of the CSA-D cross terms breaks the symmetry of the Redfield matrix elements and couples the symmetric and anti symmetric normal modes together. That is to say, following any given perturbation each individual line in any doublet will decay at a unique rate. Two types of experiments (|1H or 13C| 180^0 - τ - |13C| 90^0 - acquisition - W)n at two different field strengths lead to a set of experimental data which allow an unambiguous treatment of the three diffusion constants Dx,Dy, and Dz the two random field spectral densities ja and jx as well as $\Delta\sigma$ as variable parameters. The obtained motional parameters are in excellent accordance with those values found by Wallach when treating the quadrupolar relaxation of N and O for DMF.

The ration of J^{CSA}/J^{D} = 0.05 (50MHz) and 0.1 (75MHz) as well as the D-CSA correlation spectral densities of K^{D-CSA}/J^{D} of 0.25 (50MHz) and 0.37 (75MHz) demonstrate the importance of these terms for an understanding of the relaxation behaviour. In addition the molecular dynamic of the methylgroups has been treated, including anisotropic overall tumbling and internal motion of the molecule. Assuming the methylgroups as fully dipolar relaxed and treated as A_3X spin system leads for the cis-methyl group (with respect to the proton) to an internal rotation described by a diffusion constant D_r of 1.5 10^{11}/s, whereas the trans-methyl group ends up with a D_r of 6.8 10^{12}/s.

Pulse ESR and molecular motions

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Three pulse methods will be discussed:

1. It is possible to estimate the correlation times $\tau_c < 10^{-5}$s from the phase relaxation measurements in two-pulse electron spin echo (ESE). The results of theoretical and experimental analysis of CH_3 groops rotation in nitroxide radicals are given.

2. The pulse saturation-recovery with ESE signal detection has a wide range of possible τ_c detection up to 10^{-2}s. The data for $\dot{C}H_2$-groop rotation in $\dot{C}H_2CO_2^-$ radicals and for CH_3-groop in $CH_3\dot{C}HCO_2^-$ radicals will be discussed.

3. Then in the pulse saturation-recovery technique a pulse H_o rapid stepping is added, that gives the possibility to analyse redistribution of pulse saturation over the ESR spectrum. The latter arises due to the anisotropic motion of radicals. This type of saturation-recovery spectroscopy is applied to the nitroxide spin probes and spin labeles.

Relaxation of spin S=3/2 in the doubly rotating tilted frame

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For spin quantum number S=3/2 the time-evolution of the density operator under spin-locking is described. It is shown that due to $T_{1\rho}$ relaxation outside the extreme narrowing limit triple-quantum coherences are excited. An experiment is proposed to monitor these coherences. The corresponding signal evolves according to a simple relaxation function which is suitable for fitting. Some experimental results are presented.

Nuclear Spin Relaxation in Liquids Due to Interaction with Paramagnetic Ions Having Anisotropic g-Tensors

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Paramagnetic ions are known to make important contributions to the spin lattice $\left(T_{1M}^{-1}\right)$ and the spin-spin $\left(T_{2M}^{-1}\right)$ relaxation rates of nuclei in the vicinity. It has been recognized for some time that measurement of spin relaxation rates of nuclei in the proximity of paramagnetic ions is an attractive method for the determination of distances of these nuclei with reference to the cation such as Co(II) or Mn(II)[1]. The most commonly used form of the theory[2] (due to Solomon, Bloembergen and Morgan) assumes that the g-tensor of the paramagnetic ion is isotropic. Sternlicht has taken into account axially symmetric g-tensor[3]. However in many complexes of biomolecules such as enzyme bound substrates, the magnetic environment is expected to be highly anisotropic. With this fact in mind, we have derived[4] expressions for T_{1M}^{-1} and T_{2M}^{-1} for a general anisotropic g tensor, using the density matrix method. These rates have a strong dependence on the orientation of the ion-nucleus vector in the principal axis system of the g-tensor. The derivation will be presented and the deviations of the relaxation rates with reference to the corresponding rates for an isotropic g-tensor will be numerically illustrated for some typical paramagnetic metal-complexes.

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DEUTERIUM NMR AND MOLECULAR DYNAMICS IN SOLIDS

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Studies of deuterium relaxation gain much of their power by virtue of the fact that the dominant (quadrupolar) mechanism is free of many-body complications, being governed instead by the rotational motion of single particles. In many cases of practical interest, molecular motion in solids is fast enough to permit description of deuterium quadrupolar relaxation in terms of motional narrowing theory. For these cases, traditional theoretical approaches involve constructing a physical model which leads to formulae for the time dependent orientational probability distribution function, followed by calculation of relevant correlation functions by means of ergodic replacement of time averages with space averages. In this paper an alternative approach is explored, based on direct evaluation of correlation functions constructed from molecular trajectories calculated by Newtonian dynamics. Thus, the effective correlation times which appear as free parameters in typical motional models are replaced by parameters which purport to describe intermolecular forces and potentials.

Experimental data will be presented for perdeuterated normal alkanes trapped in urea inclusion compounds. Measurements of individual spectral density parameters $J_1(\omega_0)$ and $J_2(2\omega_0)$ as a function of temperature, field strength, and crystal orientation provide a suitable data base for stringent tests of molecular dynamic simulations. The alkane molecules are confined by the walls of the urea host lattice, and they rotate rapidly about their long axes. The rapid motion, combined with the fact that interactions between alkanes in neighboring channels can be ignored, permits relatively sophisticated simulations to be carried out with moderate computational effort.

DEUTERIUM LINESHAPES AND MOLECULAR MOTION IN INCLUSION COMPOUNDS

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Deuterium quadrupole echo NMR spectroscopy is highly useful for studies of the structural and dynamical properties of a wide variety of solid materials. Work in our laboratory during the last few years has focused on studies of inclusion compounds such as those based on urea, cyclodextrin and other organic compounds as well as intercalates with uranyl hydrogen phosphates. Examples of this work will be presented. In addition, the effect of dipolar couplings on the deuterium quadrupole echo lineshapes will be discussed.

DYNAMIC BEHAVIOR OF COMPARTMENTALIZED WATER IN CROSS-LINKED GELS WITH VARIOUS PORE SIZES

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INTRODUCTION

Recently, relaxation times of water proton in biological tissues have made a great contribution for constructing the anatomical images in MRI and also for T2-tissue characterization in vivo and in vitro. T1 and T2 reflect environment of water and interaction of water with surrounding tissues or biological macromolecules. The biological materials, however, are complex and heterogeneous. The observed T1 and T2 are generally affected by all those factors. It is important to investigate each factor individually which has an influence upon the relaxation rate and to estimate the existing state of water in tissue and specificity of the tissue itself from T1 and T2. In the present paper cross-linked polymer gels are used as model compounds of tissues and nuclear relaxation mechanism of compartmentalized water in various pore sizes were discussed. Moreover, correlation time of rotational diffusion of spin probe in the same gels was measured by ESR method. Dynamic behavior of compartmentalized water will be totally discussed from NMR and ESR data.

EXPERIMENTAL

Sepadex gels with various pore sizes (G10, 15, 25, 50, 75, 100) were used. The polymer beads were soaked by distilled water or aqueoussolution of D-Tempone little by little. The water content ranged from 30 to 70% of the total weight. Relaxation measurements were carried out by PC-120(Bruker) NMR spectrometer. T1 and T2 were calculated from 20 and 169 data points by IR and CPMG methods, respectively. T1 and T2 were analyzed by multi-exponential analysis. Temperature of measurement was changed from 5 to 50 degree of centigrade.

RESULTS AND DISCUSSION

Water in G25 gel with 50% of water content showed a peculiar property both in nuclear relaxation mechanism and in rotational motion of spin probe, which is different from water in the other gels with larger pore size than G25. That is, 1) proton exchange is dominant in the relaxation process and is superior to dipolar interaction and 2) thermal motion of water is rapid in G25 gel, although pore size of G25 gel is smaller.

The authors reported that water compartmentalized by the network structure of G25 gel remains unfrozen during cooling to -50C and crystallizes at about -12 C during subsequent warming. It is interesting that the specific dynamic properties mentioned above occur under the same codition that the thermal abnormality of water occurs.

Influence of Lateral Diffusion on the Lineshape of Spin Labels in Lyotropic Systems.

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In amphiphilic systems with curved aggregate surfaces, e.g. micellar solutions, hexagonal and cubic liquid crystalline phases, aggregate tumbling and/or lateral diffusion around the curved aggregate surfaces often occur at a rate which is in the motional narrowing regime on the NMR time-scale. However, these processes occur in the intermediate motional regime on the ESR time-scale. On the other hand, local fast molecular motions in the amphiphilic aggregates are usually in the motional narrowing regime both on the NMR and the ESR time-scale. In the present investigation, results from spin labeling of micellar and cubic isotropic, lamellar and hexagonal phases are discussed. The lineshapes of dissolved probe molecules are analyzed by means of the "two-step model", earlier developed in NMR contexts, and standard computer programs for simulations of ESR lineshapes. This procedure permits an evaluation of the slow correlation time of the label, τ_c^s. From this latter quantity it is possible to estimate the size of aggregates formed by different types of amphiphilic molecules. Finally, the difference in the lateral diffusion rate of amphiphilic molecules and its implication for the lineshape of the label is discussed.

USE OF MAL-6 AND LONG CHAIN NITROXIDES IN THE STUDY OF THE STATUS OF ERYTHROCYTE MEMBRANES IN NEWBORNS

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A previous study[1] carried out on the initial reduction rate of 4-maleimido-2,2,6,6,-tetramethylpiperidine-1-oxyl (MAL-6) introduced as aqueous solution into intact or washed erythrocytes of cord and 4-day blood has shown a significantly slower reaction rate of the radical in the cord blood as compared to that of 4-day-old infants and adults.

In order to study dynamical and structural changes underwent during the aging of the newborn erythrocytes, the same protein -SH-specific nitroxide MAL-6 was used in the present work with ghost membranes. A lower availability of -SH mobile groups from membrane proteines was demonstrated in cord blood with respect to 4-day and adult blood, with resultant lower flexibility of the cord ghosts. This was checked by the evaluation of the W/S ratios between the ESR spectrum intensities of weakly immobilized -SH (W spectrum) and of strongly immobilized -SH groups (S spectrum), according to the procedure given by Butterfield.[2] Computer simulation of the ESR total absorptions of both type of spectra allowed to get information on the motional properties of the radical either in cord and 4-day blood. The membrane structure changes involved in the membrane physical alterations were discussed in terms of membrane proteine network.

The status of the lipid double layers of the same systems was investigated with the aid of the lipophylic doxyl derivatives of stearic acid (n-DXSA) with the paramagnetic unit located in different positions of the hydrocarbon chains. The ESR lineshapes from these nitroxides introduced in ghosts from cord, 4-day, and adult blood did not show appreciable differences as a function of the RBC aging, thus suggesting that the protection against oxidative agents in the first days of life involved the proteine network of the erythrocyte membranes of the newborn more than the lipid bilayers.

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RECENT EPR AND ENDOR STUDIES ON SOME PARAMAGNETIC MOLECULE-SOLUTE-SOLVENT INTERACTIONS

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Recent developments in the experimental technique to study disordered systems have made the method of ENDOR spectroscopy a particularly useful tool for clarifying some aspects of weak and very weak electron-nuclear interactions. Thus it is possible to find data not only about the electronic but also for geometric structure of the paramagnetic molecules as well as for molecular arrangements in several systems. In the present lecture the application of the recently developed method for ENDOR study of disordered systems will be demonstrated in the case of paramagnetic molecule-solute-solvent interactions. These specific interactions as typically are not accessible by other physical methods. The structure of the donor-acceptor complexes thus formed will be discussed on the ground of the ENDOR results and will be corelated with the mechanisms of some chemical reactions known to take part with the solute (or solvent) molecules as well as with some properties of the paramagnetic molecules appearing in the studied solvents.

AN INTERNATIONAL ORDER APPROACH TO INVESTIGATING INTRAMOLECULAR ROTATIONS BY NMR IN LIQUID CRYSTALS.

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The feasibility of extracting conformational information from the proton dipolar couplings of a solute dissolved in a nematic liquid crystal (1-6) is re-examined. In particular we discuss the possibility of obtaining information on internal order (5) and conformational distribution using a maximum entropy approach (6). Some recent work on the analysis of the proton NMR spectrum of 4,4'-dichlorobiphenyl (DCB) in the nematic liquid crystal I52 (7) where purely orientational order parameters for the rings as well as an approximate rotamer distribution have been determined will be discussed.

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Stereochemistry and Dynamic of α-Cyclodextrin Inclusion Complex with D-Tryptophan

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We report here that α-cyclodextrin (α-CD) induces nonequivalence in the proton and carbon spectra, in D_2O solution, of the two enantiomers of underivatized tryptophan (Trp). The stereochemistry and the dynamic in solution of the inclusion complex formed by α-CD and D-Trp have been investigated by ^1H and ^{13}C NMR spectroscopy.

Results obtained can be summarized as follows:

a)α-CD forms a 1:1 complex with D-Trp.

b)The aminoacid is incorporated into the hydrophobic cavity of α-CD from the benzene ring side of the indole moiety, while polar groups of the alkyl chain of D-Trp give rise to attractive interactions with external polar groups of cyclodextrin.

c)The interaction with α-CD produces no slowing down of the molecular motion of D-Trp to indicate the existence of anisotropic motion of the substrate with respect to the cavity. The weak nature of the forces involved in the interaction, as well as the simmetry of the cavity may be responsible for this independent motion which probably takes place around the sixfold symmetry axis of α-CD.

STUDY ON ESR RELAXATION OF HUMAN HAIRS AND RELATIONSHIP WITH THE HEALTH

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SUMMARY

It is paying extensively deal of attention to studying on radical of human hairs in recent years. We would like to report a preliminary studying for ESR relaxation of hair's radical in this paper. The relative values of relaxation time T_1 and T_2 for the cancer patients and healthy persons have been given.

The expression of the first derivative lineshape for the ESR signal of radical as follows:

$$Y' = \frac{16}{3^{3/2}} \cdot \frac{2(H - H_0)\gamma T_2 H_1 Y_m^{0'}}{[1 + (H - H_0)^2 \gamma^2 T_2^2 + H_1^2 \gamma^2 T_1 T_2]^2} \qquad (1)$$

Where $Y_m^{0'}$ is the maximum amplitude under the unsaturated cases; $2H_1$ is the amplitude of linearly polarized microwave field, its value is depandent on the microwave power of entrance into cavity and there is a functional relationship as follow:

$$(2H_1)^2 = K \cdot P_W \qquad (2)$$

The ratio of intensities for the ESR signal under certain microwave power with under saturated point denoted by A_{pp} :

$$A_{pp} = \frac{3^{3/2}}{4} \cdot \frac{\gamma (T_1 T_2)^{1/2}}{(1 + H_1^2 \gamma^2 T_1 T_2)} K^{1/2} \cdot P_W^{1/2} \qquad (3)$$

It is clear that the A_{pp} is linear relationship with $P_W^{1/2}$ when the microwave power is small enough, ie. $H_1^2 \gamma^2 T_1 T_2 \ll 1$, and the slope of this straight line is:

$$\frac{3^{3/2}}{4} \gamma (T_1 T_2)^{1/2} K^{1/2} \qquad (4)$$

On the other hand, the relation formula for the peak width of first derivative line, we have:

$$(\Delta H_{pp})^2 = \frac{4}{3\gamma^2 T_2^2} + \frac{4 H_1^2 T_1}{3 T_2} \qquad (5)$$

For the saturated point, we have:

$$T_2^2 = 3/(\gamma^2 \cdot \Delta H_{pp}^2) \qquad (6)$$

Measured the ΔH_{pp} in saturated point, we can obtain T_2 from Eq. (6). In addition to measure a lot of ESR signal which the microwave power is far from saturated point, we can obtain a series of A_{pp} and P_W. Plotting A_{pp} vs. $P_W^{1/2}$ as Fig. 1, the value of slope evaluated by diagram from Fig. 1, then we have got T_1 using Eq. (4). Only relative value of T_1 could be obtained, because of the constant K containning in slope is unknown. But it is not important for us to distinguish the cancer patients from healthy persons. A large number of experimental data show that the value of K depandent on the position of sample in cavity, the length of hair sample, but not on the amount of the hair sample for given spectrometer.

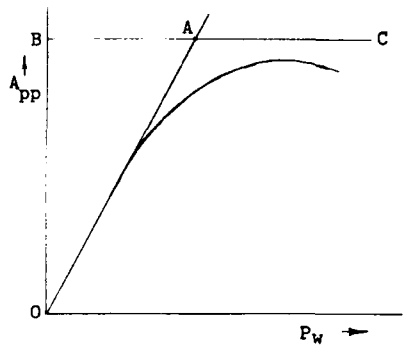

Fig. 1 Plotting the Relative Intensities A_{pp} vs Microwave power P_W.

It may be defined the point A, which is a crose point of the straight line AO and the horizontal line BC, as a threshold value. We find that if the crose point lie on the left side of point A must be patient, and if it lie on the right side of point A should be healthy person. The accuracy reach to more than 80% .

Why the relaxation time T_1 of the cancer patient hair is longer than the healthy person's. It is a very interesting problem to be further studying.

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